9

Influence of Lipids on the Bioaccumulation and Trophic Transfer of Organic Contaminants in Aquatic Organisms

Peter F. Landrum and Susan W. Fisher

9.1. Introduction

9.1.1. Sources of Contaminant Gain and Loss in Aquatic Systems

In aqueous systems, organisms are exposed to contaminants via multiple routes (Fig. 9.1). The extent of contaminant accumulation ultimately depends on the extent and mode of interaction with diverse contaminated media. The influence of lipids on contaminant uptake likewise varies according to the route by which the exposure takes place and the lipophilic character of the contaminant. Thus, it is necessary to clarify the environmental sources of contaminants for accumulation. The means by which contaminants, once accumulated, can be eliminated from an organism can also depend on organism lipid content. This elimination can be modified by the route, contaminant lipophilicity, and extent of contamination of the environmental compartment into which elimination occurs.

A few definitions will help guide the discussion. When contaminants are freely dissolved in water, uptake by aquatic organisms occurs when the contaminant is absorbed across a respiratory surface, the gills (Nichols et al., 1991, 1990), or less commonly, across the cuticular epidermis or exoskeleton (Lien et al., 1994; Lien and McKim, 1993). If uptake exceeds elimination, then *bioconcentration* has occurred. If the ultimate source of the contaminant is not water (i.e., if the contaminant is bound to some environmental medium such as sediment or is present in an organism's food), *bioaccumulation* from solid media can occur. Because some fraction of the contaminant may detach from solid media and enter the dissolved phase, some uptake of the contaminant directly from water may occur (Bruner et al., 1994a). However, it is difficult to separate the fraction of the contaminant that originates from solid media and the fraction that is directly absorbed from water; bioaccumulation, thus, is understood to include both routes of entry into an organism. Once a contaminant moves from abiotic media such as water or sediment into living organisms, the contaminants can subsequently move through the food chain when, for example, contaminated prey are ingested. *Trophic transfer* is the term that describes the transfer of contaminants between

Lipids in Freshwater Ecosystems
Edited by Michael Arts & Bruce Wainman

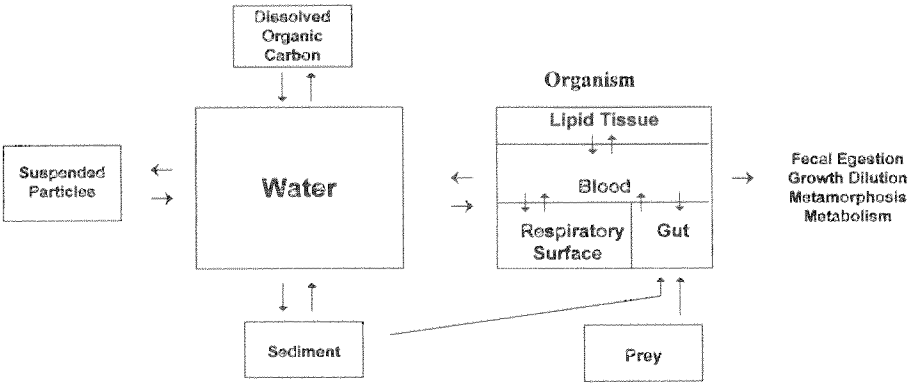

FIGURE 9.1. Sources of contaminant gain and loss for aquatic organisms. Contaminants in aquatic organisms are accumulated from both water and food. The contaminant distributes within the aquatic environment in accordance with its characteristics and those of the ecosystem between water, sediment, suspended material including particles, and dissolved organic matter. This distribution will dictate the bioavailability of the contaminant and depends on the characteristics of the interaction between the organism and phases into which the contaminant is distributed. The accumulation of a contaminant in an organism depends on the rate of exposure, the rate of distribution within the organism, and the characteristics (e.g., lipid content) of the tissues and is balanced by biotransformation and elimination processes to yield the net accumulation.

trophic levels via ingestion. A special instance of trophic transfer, known as *biomagnification,* occurs when contaminant loads increase significantly with each successive trophic level. In both cases, the primary route of exposure is thought to be from contaminated food.

Accumulated contaminants can also be eliminated from an organism. Because most contaminants are generally accumulated through passive partitioning and because the partitioning is an equilibrium process, contaminants can be eliminated from an organism's body by diffusion. The process depends on the relative affinity of the contaminant for the organism and the compartment into which the contaminant is being eliminated. Several internal physiological processes dictate the rates at that contaminants will be transported internally, deposited in lipid-rich storage, or removed via some excretory process such as fecal egestion. Contaminants that are susceptible to metabolism can be eliminated via chemical alteration. In addition, contaminant concentrations can be diluted through tissue growth even though the contaminants are still present. Such growth dilution may reduce the biological effects associated with contamination. Finally, contaminants can be lost by offloading to neonates through reproduction or, in the case of animals that undergo metamorphosis, through ecdysis. If an animal is actively bioaccumulating, then uptake must exceed loss. When the rates of uptake and loss are equal, the organism is at steady state.

9.1.2. *Organism Adiposity and Internal Distribution of Contaminants*

Although contaminants can be accumulated from several environmental compartments, once the contaminants are bioaccumulated, organism lipids become the dominant force controlling the dynamics of distribution and the manifestation of toxic effects. Lipids in organisms make up a significant portion of cell membranes, are particularly concentrated around neurons, and are the fundamental energy stores for organisms. Thus, lipids serve as membrane barriers to accumulation and distribution of contaminants, the sites of toxic action where changes in membrane function are disturbed by contaminant interaction, and the ultimate storage site for contaminants that have high lipid solubility. The interaction of contaminants with lipids depends, in large part, on the ability of the contaminant to dissolve in the lipid, and contaminants that have low lipid solubility will not cross membranes except through facilitated or active transport sites (Hunn and Allen, 1974). By contrast, highly hydrophobic contaminants will be found more strongly associated with lipid membranes and storage lipids of the organism.

Initial efforts to understand the interactions of organic contaminants and lipids come from the pharmacological literature and are focused on understanding the distribution of drugs within an organism (Bickel, 1984). Contaminants that are freely dissolved in the blood are available for transport among tissues whereas those that are sequestered by blood proteins and lipids are less mobile. Further, contaminants that are highly lipophilic eventually end up in organism lipid stores away from their receptor sites for toxic action However, lipid mobilization by an organism under stress can release contaminants back to the general circulation. In addition, contaminants of differing lipophilicity differ in their ability to be assimilated because crossing the cell membranes into an organism requires the contaminant to pass through lipid membranes. Thus, differing lipid solubilities along with other molecular features such as molecular size produce differences in the rates and extent of bioaccumulation. Because of the interest in developing better drugs with more improved transfer efficiencies and rates, the partitioning of contaminants between water and representatives of lipid materials has been studied. The best small molecule found to be representative of lipid materials was n-octane-1-ol (usually referenced as octanol). The partitioning behavior between octanol and water provides a standardized reference source for partitioning that has proved very useful for evaluating the lipophilicity of contaminants (reviewed in Leo et al., 1971).

The kinetics and efficiency of transport into organisms are determined by the rate of presentation of contaminants to the membrane, the chemical activity (chemical potential) difference between the source compartment and the organism tissue, and the contaminant's resistance for crossing membranes. Because membranes are composed primarily of lipid bilayers, the ability of contaminants to dissolve into, and move through, membranes dictates part of the kinetics of transport (Hunn and Allen, 1974). The rate of exit from the membrane into the circulating fluid and transport to the site of action or storage can also significantly

dictate the rate of accumulation of lipophilic contaminants. The same factors affect the transfer of the contaminants out of a tissue. In summary, when the lipid solubility of the contaminant is high relative to its solubility in the source compartment, the contaminant will tend to pass into and accumulate in the organism.

The impact of lipids on these kinetic processes is most easily observed in their effect on the rate of elimination. Organisms with higher lipid contents exhibit elimination rates that are substantially slower than observed for leaner organisms (Van den Huevel et al., 1991; Landrum, 1988). Differences in the overall elimination rates among organisms reflect the capacity of the organisms relative to the source compartment, and decreases in elimination rate are reflected in increasing bioaccumulation. In some cases, the rate of accumulation can increase with increasing lipid content as was observed for the zebra mussel (Bruner et al., 1994a). The mechanism for this increase is tied to maintenance of the concentration gradient between the source compartment and the site of uptake. This presumes that the rate processes involved in the distribution within the organism are not rate-limiting. If distribution limitations become a significant portion of the rate process, then distribution to the storage lipid will become disconnected from the uptake rate process and lipid content will no longer be tightly coupled to uptake rate. This was clearly demonstrated with the accumulation of trifluralin in rainbow trout, in which the uptake clearance rates declined in proportion to the intercompartmental transfer rates with increasing organism size despite the increased lipid content for large fish (Schultz and Hayton, 1994).

9.1.3. Nonlipid Factors Affecting Internal Distributions

In addition to the lipophilicity of the contaminant, the size of the molecule may preclude its dissolution into the membrane and, therefore, accumulation by the organism. In the extreme case of polymers, it is clear that even hydrophobic (highly lipophilic) molecules such as polymers of polydimethylsiloxane are not accumulated and are only found on the surface of organisms (Kukkonen and Landrum, 1995; Opperhuizen et al., 1987). The failure of molecules to accumulate can also be due to binding to extracellular materials such as dissolved organic carbon (Bruggeman et al., 1984) or decreased permeability of the membrane resulting from molecular size limitations, >9.5 Å (Saito et al., 1990; Opperhuizen et al., 1985; Zitko, 1980). Both mechanisms result in reduced bioavailabilities. The effective molecular size range for interaction with lipids not only has an upper limit, at which the lipophilic membranes act as essentially impermeable barriers, but there is also a lower molecular size cutoff. At the lower end, the membranes are permeable to small un-ionized molecules. This size cutoff is <50 a.m.u. (Walter and Gutknecht, 1986). The extra permeability is not related to the lipophilicity of the contaminant but rather inversely related to the molecular size. The molecular volume dependence was attributed to the membrane properties, with the lipid behaving more like a polymer than a liquid hydrocarbon (Walter and Gutknecht, 1986).

Within the effective molecular size range, this relative solubility between the source compartment and the organism's lipid is a useful predictor of the potential extent of accumulation. For contaminants that contain ionizable functional groups, the pK of the contaminant will influence the final storage site in the organism (Hunn and Allen, 1974). For instance, pentachlorophenol (PCP) ionizes and has a pKa of 4.74 (Westhall, 1985); its penetration into the organism requires, in general, that the un-ionized molecule penetrate the membrane (Stehly and Hayton, 1990), which is reflected by the apparent lipophilicity of the contaminant as reflected by apparent changes in the octanol/water partition coefficient with pH (Kaiser and Valdmanis, 1982). In the Great Lakes, where the pH is about 8, PCP would be highly ionized (approximately 0.05% in the un-ionized form) so penetration of the lipophilic membrane to enter the animal will be limited. In the case of *Diporeia,* the uptake clearance for PCP (log K_{ow} 5.01; Westhall, 1985) from Lake Michigan water was 3.74 ml $\cdot$ g^{-1} $\cdot$ h^{-1} (Landrum and Dupuis, 1990), whereas the uptake clearance for pyrene, a contaminant of similar log K_{ow} (5.2), was 131 ml $\cdot$ g^{-1} $\cdot$ h^{-1} (Landrum, 1988). Similarly, the distribution within the organism may become limited because of ionization in the circulatory fluid of the organism, which limits distribution within and, in some cases, elimination from the organism. The impact of ionization is equally important for contaminants having basic as well as acidic functional groups. The relative state of ionization will dictate the degree of penetration into the lipophilic environment of the membrane due to changes in lipid solubility of the contaminant.

9.2. Prediction of Bioconcentration and Bioaccumulation

9.2.1. Bioconcentration

Once the contaminant enters the organism and is distributed to the final storage site, the relative solubility in lipid will permit predictability. As with the distribution between the circulating fluid and the tissue, the partitioning characteristics of the contaminant between 1-octanol and the source can help predict the accumulation potential of the contaminant. This approach works as well for terrestrial organisms as it does for aquatic organisms (Kenega, 1980). For aquatic organisms exposed in contaminated water, nonpolar contaminants are accumulated in proportion to the octanol/water partition coefficient (K_{ow}) of contaminants. This was first demonstrated by a prediction of the bioconcentration of contaminants by fish. The log of the bioconcentration factor (log BCF is defined as log of the ratio of the contaminant concentration in the organism to the contaminant concentration in the water) was linearly correlated with the log K_{ow} (Neely et al., 1974). The use of this approach was further demonstrated through the work of Veith et al., (1980, 1979) and Mackay (1982) on fish. Subsequently, the bioconcentration in both invertebrates and macrophytes was examined with respect to log K_{ow} (Gobas et al., 1991; Connell, 1988; Hawker and Connell, 1986). In all cases, there was evidence of nonlinearity for large, very hydrophobic contaminants. Some of this

nonlinearity was accounted for by molecular size as stated above. Steric properties (e.g., molecular size, surface area, or configuration) of even relatively small molecules such as polychlorinated biphenyls (PCBs) can influence the relative bioaccumulation of contaminants compared with traditional log BCF $-$ log K_{ow} relationship (Shaw and Connell, 1984). In addition, there is evidence that 1-octanol becomes a less ideal solvent for larger molecules. Thus, accounting for the relative solubility in octanol removes some of the observed nonlinearity in BCF prediction (Banerjee and Baughman, 1991).

Because the contaminant lipophilicity as measured by K_{ow} and extent of contaminant accumulation are so well related, a convention developed to normalize the contaminant concentrations to the lipid contents of organisms. This technique reduced the variability between organism species and resulted in improved predictions for accumulation from water (Barron, 1990; Connell, 1988). However, cases remain that demonstrate the limitations of lipid normalization to totally account for the variation in contaminant accumulation among species. For instance, in Lake Baikal lipid-normalized BCF-K_{ow} relationships had different slopes for two fish species (Kucklick et al., 1994). Similarly, the BCFs for lake trout and white fish from Siskiwit Lake on Isle Royle exhibit significant variability even with lipid normalization; the regression with log K_{ow} was weak for pesticides, and the correlation for PCBs was even more variable (Swackhamer and Hites, 1988). In some cases, the absence of improved relationships despite lipid normalization may be due to inclusion of multiple contaminant classes in the regression (Axelman et al., 1995; Connell, 1988). Where contaminant characteristics change, the interaction with lipids also changes. Thus, even in a single species, the slopes of the relationships between lipid-normalized BCF and log K_{ow} are different for different contaminant classes (Axelman et al., 1995). Thus, predictability will depend on both the composition of the lipids and the characteristics of the contaminant. Both characteristics will contribute to an interaction that will determine the relative contaminant solubility in the organism's lipids and the ability of log K_{ow} to predict that solubility interaction. This predictability will generally be good within a species and class of contaminants (Axelman et al., 1995; Connell, 1988). However, the variance in the predicted BCF may be substantial if attempts are made to predict across species and contaminant classes (Connell, 1988). For instance, the intercepts for regressions of log K_{ow} against the lipid-normalized BCF range over an order of magnitude among organisms whereas the slopes vary from 0.844 to 1.0. (Connell, 1988). Despite the above-mentioned caveats, lipid normalization of contaminant concentrations remains the most viable and useful method of predicting BCFs and serves an important role in screening new contaminants for potential BCFs.

9.2.2. Bioaccumulation

The variance in the predicting contaminant accumulation, using such predictors as log K_{ow}, increases substantially compared with predictions from aqueous exposures when attempting to predict the thermodynamic limits for contaminated

sediment exposures. The increased variance results from the organic matrix of the sediment competing with the organism lipids for the relative solubility of the contaminant. There is a hypothesis that if a correction is applied for the amount of organic matter in the sediment, generally expressed as organic carbon, then the thermodynamic limit for bioaccumulation becomes relative to the freely dissolved contaminant concentration in the interstitial water, which is representative of the chemical activity (DiToro et al., 1991; McFarland and Clark, 1989; McFarland, 1984). According to this model, the derived biota sediment accumulation factor (BSAF), in which the concentrations in the sediment are carbon-normalized and those in the organism are lipid-normalized, should be invariant with log K_{ow}. The theoretical thermodynamic partitioning relationship between the sediment organic matter and the organism lipid leads to an estimated constant value of 1.7 (McFarland and Clark, 1989). This value represents the relative capacity of organism lipid and sediment organic matter on a unit mass basis and should not vary with log K_{ow}. In studies that have measured BSAF among organisms and sediments, the variance can exceed 100-fold between the lowest and highest values even for the same contaminants (Brannon et al., 1993; Lee, 1992; Lake et al., 1990). The magnitude of this range was suggested to result from an absence of true equilibrium/steady state for some of the data.

The difficulty appears to be in accounting for the bioavailability of the organic contaminants in the sediment matrix. If the uptake clearance from the sediment (the amount of sediment cleared of contaminant per mass of organism per hour) is used as a measure of bioavailability, simple normalization to the amount of organic matter in the sediment is inadequate to explain all the observed variance by a factor of 10 (Landrum and Faust, 1994). Further, bioavailability is not even the same for contaminants of similar hydrophobicity from a single sediment (Harkey et al., 1994a; Landrum and Faust, 1991). This change in bioavailability appears to be driven in part by differences in the distribution of contaminants among the varying particles within the sediment matrix (Kukkonen and Landrum, 1996; Harkey et al., 1994b). Further, these distributions do not always correlate with the amount of organic carbon in a particular sediment fraction (Kukkonen and Landrum, 1996; Harkey et al., 1994b). Thus, it is not surprising that the relationship between sediment concentration and bioaccumulation is not regular even when normalized for organic carbon and lipid. Prediction among sediments will likely require additional factors to better account for the interaction of the contaminant with the sediment.

9.3. Factors Affecting Prediction

9.3.1. Methods for Measuring Lipid Content

Among the factors that affect the ability to predict either bioconcentration from water or bioaccumulation from sediments is the method used to measure the lipid content of the organism. Lipids have been extracted through the use of several

organic solvent combinations. Most frequently, the solvent chosen for the lipid extraction is determined by that which most efficiently extracts the contaminant of interest from the various matrices. This often leads to incomplete extraction, sometimes in excess of 50%, of the total lipids and is generally a failure to extract the polar lipids (Ewald, 1996). Further, the solvents specifically used for lipid extraction sometime fail to completely extract the contaminants, particularly in leaner organisms with a difference of nearly 40% in some cases (Ewald, 1996). There has been little effort to intercalibrate the relative extractability of the various solvent systems with a more conventional system, chloroform:methanol, to extract lipids (Bligh and Dyer, 1959; Folch et al., 1957). These extraction differences may lead to substantial differences in comparing the lipid-normalized bioconcentration across studies and among species of varying lipid composition. We suggest that the substantial variability in lipid extractability among data sets could be eliminated by standardizing to the Bligh-Dyer extraction scheme, which uses a chloroform:methanol extracting solvent and is known to extract both polar and nonpolar lipids (Randall et al., 1991). It would not be necessary for all studies to use a single extraction technique, but there should be an intercalibration between the extraction solvent of choice and the Bligh-Dyer scheme. This would permit calculation to common units. This scheme can even be adapted for the measurement of lipid content for small sample masses (Gardner et al., 1985).

9.3.2. Lipid Composition and Bioaccumulation

The relative lipid compositions among species also contributes to the observed variation in bioaccumulation potential. The generally held view is that neutral storage lipids are the most important class for the bioaccumulation of nonpolar contaminants. Thus, species with higher neutral lipid fractions would presumably accumulate higher contaminant concentrations on a lipid-normalized basis. However, the evidence for the role of lipid composition is limited. Several studies have focused on the relative extractability of the contaminant of interest and the corresponding lipid to suggest the role of lipid composition on bioaccumulation (Ewald, 1996; de Boer, 1988; Schneider, 1981). Another approach examining the role of lipid composition to compare across species and contaminant characteristics used different portions of the lipids as normalizing factors. When this is done, prediction of bioaccumulation was better related to total lipid content than to any one lipid fraction (Kucklick et al., 1996; Stange and Swackhamer, 1994).

The relative distribution of the moderately lipophilic herbicide triallate was investigated by using autoradiography (Arts et al., 1995) and mass spectrometric techniques (Arts et al., 1996) and showed that the contaminant resides primarily in the storage lipids and in the highly lipophilic nervous system tissues. Although the triallate was found in storage lipids as expected, the bioaccumulation did not always correlate with the triglycerol content of the amphipods (Arts et al., 1995). This suggests a role for other lipids or other storage sites in some species that were not detected by the above techniques.

Some experiments have examined this issue more directly. One such study extracted lipids from phospholipid-rich organisms and from triglyceride-rich organisms. The extracted lipids were coated onto filters, and the relative partitioning onto the filters was determined. The data indicated that the filters coated with triglyceride-rich lipids accumulated nearly twice the amount of tetrachlorobiphenyl compared with filters containing lipids with 20% or more phospholipids (Ewald and Larsen, 1994). From this more direct study, the bioaccumulation by biota was deduced to vary with triglyceride composition of the species.

In another study, the two lipid types, buoyant nonpolar and polar membrane-associated lipids, were separated by physical means, and the corresponding accumulation of contaminant in each phase along with the corresponding lipid composition of each phase were examined. On a lipid-normalized basis, both phases contained essentially the same concentration of the nonmetabolized contaminants, suggesting that the polarity of the lipid when it exists in the organism does not dramatically affect the accumulation of nonpolar contaminants (Gardner et al., 1990).

The ability to accurately determine the influence of differing lipid compositions contaminant bioavailability is not completely resolved, and new approaches to study the issue are required. An additional interesting finding of the Gardner et al. (1990) study was a dynamic imbalance (differences in normalized lipid concentrations) among the lipid pools that continued for a relatively long time period (>24 h) in mysids. Thus, before comparing among lipid pools, it is critical that adequate exposure time be allowed to ensure that the pools are dynamically in balance (i.e., have equal chemical activities [lipid-normalized contaminant concentrations] among the lipid pools). If this is not the case, the analyses may artificially indicate that there are differences in the contaminant distribution relative to lipid content when, in fact, the organism is out of steady state relative to the distribution among the various pools. The absence of balance among the lipid pools is more likely to occur for larger animals in which the distribution processes require longer times before pools attain equal chemical activity.

9.4. Mimicking Bioconcentration with Semipermeable Membrane Devices

With the recognition that the lipid content of organisms drives the bioaccumulation of nonpolar contaminants, attempts have been made to develop nonbiological surrogates. These surrogates would directly sample the chemical activity of systems without the difficulty of maintaining live organisms or accounting for processes such as biotransformation that complicate the interpretation. Early efforts used solvent-filled dialysis membranes or hexane "droplets" as surrogates for accumulation of contaminants (Södergren and Okla, 1988; Södergren, 1987). This early work produced encouraging comparisons between the accumulation by nonbiological surrogates and organisms. However, there were difficulties with the

hexane-filled bags and with the dialysis tubing. The hexane tended to dialyze out of the bags, and the dialysis membranes degraded over time. More recent approaches have used thin polymeric film membrane bags with a lipid material for filler (Huckins et al., 1990a) and an improved method for contaminant recovery from these samplers (Huckins et al., 1990b). Because these semipermeable membrane devices (SPMDs) are filled with lipid materials, they should more accurately reflect the contaminant capacity of lipids in organisms. Further, unlike solvents, the large molecular size of the lipids precludes their diffusion out of the sampler into the water.

The properties of these samplers have been well characterized by deploying them alongside caged organisms including both fish and bivalves (Ellis et al., 1995; Prest et al., 1992). In both cases, the SPMDs performed well in sampling the environment for nonpolar organic contaminants. In one study, the results of the SPMDs were compared with another method that measured concentrations in ultrafilter permeates (Ellis et al., 1995). The SPMDs gave equivalent results with the ultrafilter permeates in indicating the amount of contaminants bioavailable for aqueous exposures. However, caged (channel catfish) and feral fish (carp and sauger) residues exhibited fewer contaminants. The differences between caged and feral fish may be due to mobility of the feral population reflecting differing sources, differences in species, and enhanced metabolism in the feral organisms. In general, the SPMDs had higher concentrations of contaminants compared with the fish. This may reflect more rapid accumulation, the absence of biotransformation, and/or rapid elimination of the contaminants from fish (Ellis et al., 1995). When contaminant concentrations in the samplers were compared with caged bivalves (*Corbicula fluminea*), there were again differences between the mussels and the SPMDs. In general, the SPMDs sequestered a wider range of contaminants, and there were differences in the distribution between the clams and the SPMDs. Further, the clams sequestered greater concentrations than the SPMDs, which may be the result of greater uptake rates and additional pathways such as accumulation from food (Prest et al., 1992). It is clear that SPMDs can take a long time to come to true equilibrium, and this can be further modified by biofouling in aquatic systems. However, these devices hold the potential to provide a good baseline for aqueous exposures. Because they are passive storage devices, they will never reflect the active processes that govern the uptake and loss of contaminants by aquatic organisms. This also serves to suggest that although bioconcentration and bioaccumulation, even when evaluated at steady state, may be proportional to the storage capacity (lipid content) of the organism, neither may reflect the chemical activity of a given contaminant in the system. Both bioconcentration and bioaccumulation depend on the toxicokinetics that are dictated by chemical and biological processes affecting contaminant exposure and loss and the available contaminant sources.

9.5. Toxicity and the Role of Lipid

9.5.1. Release of Sequestered Contaminant During Metabolism

Because persistent organic contaminants accumulate in lipid material, alteration of the lipid pool can alter the toxicity of contaminants. For example, when lipid-rich organisms such as salmon are starved to reduce the total lipid, stored toxins are also released, which can produce deleterious results (Ewald, 1996; Bickel, 1984). Further, organisms that are rich in lipid can sequester toxic contaminants in storage sites, thereby removing the toxin from the site of toxic action (Geyer et al., 1994, 1993; van den Huevel et al., 1991; Bickel, 1984). Thus, the relative storage capacity of organisms needs to be considered not only for bioaccumulation but also for toxicity. Normalization to the amount of lipid in the organism is suggested to reduce this variation when comparing toxicity values for neutral organic contaminants among species (Geyer et al., 1994). Normalized contaminant concentration data lead to the idea of the survival of the fattest. This concept has been incorporated into a model of exposure and toxic response for narcotic contaminants. Not only will the fattest organisms have additional storage capacity, the higher fat content will impart additional energy reserves to help the organism weather stresses (Lassiter and Hallam, 1990).

The significance of lipid content and composition will increase as attempts are made to establish residue effects concentration in organisms. Residue effects concentrations are the measured concentrations of contaminant, on a whole-body basis, that are equated with a toxic response. Residue effects concentrations are under development in an attempt to move aquatic toxicology from the use of the external environment as a measure of exposure to the internal dose as the exposure measurement (McCarty and Mackay, 1993). There have been several studies demonstrating the reduction in overall range of doses required to produce mortality, particularly for nonpolar narcotics (e.g., polycyclic aromatic hydrocarbons [PAHs], chlorinated benzenes, PCBs), compared with the use of external concentrations (e.g., for fish, as reviewed by McCarty and Mackay, 1993; for amphipods, Landrum et al., 1994, 1991, and Landrum and Dupuis, 1990; for daphnids, Pawlisz and Peters, 1993a,b). The remaining variance can be attributed to three potential causes: the relative capacity for a contaminant of one species or group of organisms over another (i.e., differences in lipid content), the inherent difference in the sensitivity of one species or portion of the population versus another for the contaminant, and the relative biotransformation capability of different organisms. For contaminants that act as narcotics and are not readily biotransformed, differences in the sensitivity among and within species can be largely accounted by adjusting for the lipid composition and content of the organisms. For example, the variation in the lethal body burden (residue effects concentration) in fathead minnows exposed to chlorinated hydrocarbon contaminants was reduced 50% by accounting for the minnows' lipid content (van Wezel et al., 1995). Specific

comparisons among species and the role of lipid content on residue effects concentrations have yet to be fully explored. However, based on the intraspecies role of lipid and the general understanding of the storage of toxins and their impact on the toxic response (see above), it is clear that variation in the storage lipid capacity among organisms will help explain the variance among species when comparing residue-based effects.

9.5.2. *Lipids and Membrane Narcosis*

The direct interactions of contaminants with membrane lipids can result in narcosis. Two reviews on this subject (van Wezel and Opperhuizen, 1995; Mullins, 1954) suggest that the function of the lipid membrane becomes impaired when a sufficient molar volume of contaminant becomes dissolved in the membrane. The in vivo membrane burden of toxicant that produces narcosis is 40–160 mmol · kg^{-1} lipid, and this range corresponds to approximately 3 ml · kg^{-1} on a volume basis (Mullins, 1954). The change in membrane function is thought to occur because the ion permeability of the membranes increases due to an increase in fluidity of the lipids with the solubilization of contaminants (van Wezel and Opperhuizen, 1995). Such changes in membrane fluidity have been demonstrated in the presence of the narcotic benzyl alcohol through the use of nuclear magnetic resonance (NMR) (Ma et al., 1992). These NMR studies demonstrated that the thermodynamic character of lipid membranes changes with the introduction of a narcotic.

9.5.3. *Effect of Toxins on Lipid Metabolism and Function*

The incorporation of modified fatty acid molecules, such as chlorinated fatty acids, into the lipid biomolecules and subsequently into membrane structures is a newly recognized problem. The presence of chlorine in the fatty acid moiety causes a bend in the molecule similar to that produced by a double bond. Changes in chemical character and molecular conformation may or may not be recognized by the biochemistry of the organism. When these chlorinated fatty acids are incorporated into lipid bilayers, the membrane character may be altered. Further, incorporation of lipids containing chlorinated fatty acids into storage lipids should not initially affect the organism, but when these are mobilized for energy, there may be less energy available (Ewald, 1996). In highly polluted areas, up to 1% of the total fatty acids in an organism may be chlorinated (Håkansson et al., 1991).

The exposure to contaminants can also directly affect the lipid content, either through effects on lipid metabolism or by increasing the overall stress, thus increasing catabolism with resultant reductions in lipid. However, the exposure to chemicals need not always result in reductions in lipids. Rainbow trout (*Oncorhynchus mykiss*) exposed to heptadecane not only sequester the contaminant in the lipid stores but metabolize the heptadecane to fatty acids that become incorporated into the neutral and phospholipid fractions of the organism (Cravedi and Tulliez, 1986).

More often, however, exposure to contaminants results in a reduction in lipids. For instance, when rainbow trout were exposed to PCP, the surviving organisms at the high dose showed significant reductions in total lipids (van den Huevel et al., 1991). Other similar examples exist for exposures to both organic and inorganic contaminants. For example, the accumulation of lead in fish yielded reductions in total lipids, phospholipids, and cholesterol. This was accompanied by an increase in lipase and free fatty acids (Tulasi et al., 1992). Both of these effects were observed during the preparation for reproduction. Organic pesticides, γ-BHC and malathion, were observed to affect lipid metabolism during the vitellogenic phase of the annual reproductive cycle in the catfish *Clarias batrachus* (Lal and Singh, 1987). These contaminants affected both nonpolar lipid and phospholipid metabolism. In particular, these pesticides inhibited the esterification of free fatty acids to acyl glycerides and also affected their mobilization from liver to gonads (Lal and Singh, 1987). Thus, the impact of contaminants on lipid metabolism, particularly in preparation for reproduction, provides some insight into mechanisms for reproductive impairment of fish and presumably to other aquatic organisms exposed to environmental contaminants.

9.6. Relevance of Food Chain Transfer to Bioaccumulation

9.6.1. Relevance of Trophic Transfer to Bioaccumulation

Previous sections have dealt primarily with the uptake of contaminants directly from water and the role of lipids in determining bioconcentration. However, living organisms can also accumulate contaminants via consumption of contaminated food. Dietary transfer of contaminants has been a controversial subject for several decades. A large number of investigators have argued that uptake of contaminants from dissolved form in water eclipses accumulation from any other source and, thus, can be considered the primary source of contaminant exposure in the aquatic environment (Shaw and Connell, 1986; Bruggerman et al., 1981; Chiou et al., 1977; Moriarty, 1975). Because there are tremendous political and policy ramifications that stem from resolution of this issue and because the role of lipids in bioaccumulation takes on several added dimensions if accumulation from food is significant, the major points of argument are presented here.

Identification of bioconcentration as the most relevant route of contaminant exposure stems largely from the observation that uptake of contaminants from water is very rapid and can quickly generate significant body burdens (Fisher et al., 1993; Reynoldson, 1987). Because bioconcentration is a partitioning phenomenon between an aqueous phase and a lipid phase, movement of the contaminant from water into the organism is driven by the lipid content of the organism and is usually predictable from log K_{ow} as described above. Short-term laboratory assays are effective in measuring bioconcentration. However, in aquatic systems, most of the contaminant load is retained in sediment (DiPinto et al., 1993). Thus, it is highly likely that slower transfer processes such as accumulation from sediment

or from food are more relevant to long-term ecosystem dynamics. Dietary accumulation requires contaminant desorption from a lipophilic site in food into water in the gut, then subsequently to a lipophilic phase in the intestinal membrane. This two-phase process is less kinetically favorable and less predictable than a simple water-to-lipid partitioning. In addition, long-term assays are needed to quantify bioaccumulation and trophic transfer. However, it now appears that bioaccumulation is a dominant process at lower trophic levels, where benthic invertebrates play an ecologically pivotal role in removing contaminants from storage in sediments and funneling the contaminants into aquatic food chains (Landrum and Robbins, 1990; Swartz and Lee, 1980). Subsequent trophic transfer of contaminants following lower-tier food chain introduction may account for as much as 90–99% of the contaminant load in top predators (Thomann, 1989; Rubenstein et al., 1984; Thomann and Connolly, 1984). It is now acknowledged, in a large number of studies that have used organisms as diverse as invertebrates and fish, that diet is the primary source of contaminant for highly lipophilic (log $K_{ow} > 5$), nonmetabolized chemicals (Landrum et al., 1991; Rasmussen et al., 1990).

Establishing the reality of trophic transfer of contaminants in food chains has important implications for hazard assessment. For example, if the primary source of contaminant accumulation is not water, as suggested by the proponents of bioconcentration, then merely removing contaminants from the water column cannot necessarily be equated with a reduction in hazard. Exposure of living organisms to contaminants will continue as long as contaminants are present in any biologically active medium. In addition, contaminants can move from aquatic to terrestrial environments via consumption of contaminated prey. Food chain length is also important when trophic transfer is accompanied by biomagnification. Lengthening the food chain by introducing species can exacerbate this problem (Rasmussen et al., 1990). Thus, exposure of humans and fish-eating wildlife is, thus, an ongoing health concern (Rasmussen et al., 1990).

9.6.2. Role of Lipids in Food Chain Accumulation

9.6.2.1. Fugacity Model

In theory, the type and amount of lipid in an animal should determine the extent to which a lipid-soluble contaminant will accumulate at each trophic level in a food chain in relation to the chemical activity of the contaminant in the source compared with the organism (the sink) in question. However, to accurately assess the contaminant movement via ingestion, it is necessary to account for lipid levels in both the food (the source of contamination) and the predator (the sink). Additionally, several factors, which do not directly relate to lipid levels, are potentially very important in determining food chain accumulation. These include feeding rates, digestibility of prey, gut retention time, length and morphology of the digestive tract, the rate of fecal egestion, and the growth rate and age of the organism (Sijm et al., 1992; Clark and Mackay, 1991; Thomann, 1989; Serafin, 1984). These factors may obscure the role of lipids in trophic transfer.

The complexity of assessing food chain accumulation calls for an organizing principle that can cull factors of little relevance and highlight processes or factors that are germane. The fugacity concept is particularly useful for discerning the role of lipids in trophic transfer.

The term *fugacity* was originally used to describe the thermodynamic tendency for a gas to escape from one phase and to enter another. The term comes from the Latin "fugal" to flee. Fugacity (f) is defined in units of pressure (pascals or Pa) and is related to chemical concentration, C (mol · m^{-3}), through the fugacity capacity, Z (mol · m^{-3} · Pa^{-1}) where

$$Zf = C \text{ or } f = C \cdot Z^{-1}$$

Thus, fugacity increases linearly with contaminant concentration. The fugacity capacity is specific to a particular compartment and assesses the ability of that compartment to hold the chemical (i.e., prevent it from escaping). The fugacity capacity of a compartment can be considered analogous to the heat capacity of a material. The fugacity capacity is effectively half a partition coefficient and is often determined empirically (Mackay, 1991). A chemical will always move from high to low fugacity unless active transport is occurring (Gobas and Mackay, 1987). At equilibrium, the fugacity in all compartments will be equal (Landrum et al., 1992), and flux, N (mol · h^{-1}), of a chemical between phases will be zero.

The simplest fugacity-based contaminant accumulation models are bioconcentration models that assume lipids are the driving force in accumulation. The fugacity approach to determining bioconcentration is analogous to determining a lipid-based partition coefficient. The lipid-normalized BCF is defined as the ratio of the concentration of contaminant in organism lipid to the contaminant concentration in water at steady state:

$$\text{BCF} = (C_{\text{organism lipid}}) \cdot (C_{\text{water}})^{-1}$$

Similarly,

$$(C_{\text{organism lipid}}) \cdot (C_{\text{water}})^{-1} = (Zf_{\text{organism lipid}}) \cdot (Zf_{\text{water}})^{-1}$$

Because at equilibrium, f is equal in all phases,

$$\text{BCF} = (Z_{\text{organism lipid}}) \cdot (Z_{\text{water}})^{-1}$$

In fugacity terms, the lipid-normalized BCF can be calculated simply as the ratio of the two fugacity capacities for the respective lipid and water phases.

Although the transfer processes involved in trophic transfer are more complex than in bioconcentration, simple fugacity-based (thermodynamic) models can still be used to describe accumulation. Additionally, parameters can be included in the model to account for uptake from water as well as accumulation from food. For instance, Thomann (1989) constructed a simplified food chain consisting of phytoplankton, zooplankton, small fish, and predatory fish. Uptake of contaminants by algae consisted of simple lipid-normalized bioconcentration. However, accumulation in planktivores and predators included terms describing not only uptake from water but assimilation from food as well. The latter were all lipid-normalized

to correct for variability in accumulation due to lipid content. This model works well but is very dependent on the concentrations in the lower food web that are predicted from equilibrium partitioning theory (Thomann, 1989).

9.6.2.2. Mechanism for Trophic Transfer

The fugacity-based approach to understanding trophic transfer has yielded important information about the mechanisms of trophic transfer and their relationship to lipid levels. One implication of the fugacity model, for instance, is that the fugacity of the food must be higher than the fugacity of the organism for trophic transfer to occur. This can occur if the predator is kinetically limited in achieving steady state compared with the prey. Then the prey will be at a higher thermodynamic chemical potential than the predator. Such a situation can easily occur with large growing predators such as large fish that may never attain the thermodynamic chemical potential of the system, and thus transfer of the contaminant will continue down a chemical activity gradient. It may also be the case that the prey is exposed to sources not available to the predator (e.g., the prey come from the sediment and the predator only experiences the overlying water). This could readily occur because fine sediments are focused into depositional areas that are often out of thermodynamic equilibrium with the overlying water.

More often, however, the predator and prey may be at the same thermodynamic potential relative to the major source (e.g., water). How then can bioaccumulation occur from the prey to the predator? By empirical observation, most organisms in a food web appear to have similar fugacities (Gobas et al., 1993a). Thus, the required lipid–water–lipid partition (chemical activity gradient) that is needed to move a contaminant from ingested food into an aqueous phase and then back to a lipid phase in the organism would seem to be absent. Thus, if trophic transfer were to occur, contaminants would have to move without or against a fugacity gradient. In the absence of active uptake, there is no mechanism to account for this.

A focus on lipid and its influence on contaminant transfer has been invoked to explain this apparent contradiction. When contaminated food is ingested by an organism, the contaminants move into the gastrointestinal tract (GIT) in association with lipids. Once present in the gut, digestion begins to take place. Lipids are dissociated from the bulk of ingested material, acted on by specific enzymes, and transported across the gut wall via specific lipoprotein carriers. Two hypotheses can be invoked for contaminant transfer: (1) the contaminants may stay associated with the lipid material and move with the lipids actively, and (2) the contaminants are left behind while the lipids are digested and absorbed. This has two important consequences: (1) there is a smaller food volume remaining in the gut, resulting in a momentary increase in the contaminant concentration; and (2) the food remaining in the GIT has lost part of the lipid component that sequesters contaminants. Thus, the ability of the remaining food to dissolve the contaminant is reduced and the fugacity capacity of the food goes down, causing the fugacity of the food to become higher than that of the organism. The increase in concentration and fugacity of the contaminant in the food relative to the organism causes the contaminants to passively diffuse food across the gut wall into the organism. Thus,

net movement of the contaminant between predator and prey, which appear to have equal fugacity, becomes possible (LeBlanc, 1995; Gobas et al., 1993a; Thomann, 1989). In either mechanism, lipids become the driving force for the bioaccumulation of contaminants from food.

Because lipids are the driving force behind trophic transfer (Gobas et al., 1989), it is important to account for the influence of both lipid in the food and lipid in the organism, which may have contrasting effects. Organism lipid levels have been previously discussed. How will contaminant transfer from a high-lipid food differ from a low-lipid food, for instance? According to the fugacity concept, if the primary phase responsible for dissolving the contaminant is lipid, then the fugacity capacity of high-lipid food should be high, resulting in low fugacity at a given food concentration. In other words, less contaminant should move into the consumer's tissues from a high-lipid food than a low-lipid food. In a single study of this issue, uptake of most hydrophobic contaminants from a low-fat diet was much higher than from a high-fat diet (Gobas et al., 1993b). Although the fugacity-based explanation for this observation may be correct, in the case in which the fugacities in the two food sources are equal, it may be true that the digestibility of the high-lipid food is decreased and fugacity undergoes a greater elevation for the low-lipid food during the transit through the GIT. Discerning the role of food lipid in trophic transfer has been identified as a critical research need (Clark and Mackay, 1991).

9.6.3. Factors Affecting Trophic Transfer

9.6.3.1. Assimilation Efficiency

Trophic transfer of contaminants is frequently assessed and quantified by using an assimilation efficiency (AE) as the critical measurement. At its most basic level, %AE is simply the ratio of the contaminant retained in the consumer's tissues compared with the contaminant ingested (Harkey et al., 1994b). A question in anticipating how AE values will vary in nature is, how will AE be affected when tracking the fate of contaminants with different hydrophobicities? Measurement of the AE for organisms can be extremely difficult unless the food source can be isolated and the exact contaminant concentration determined. For many selective feeding organisms, such as benthic amphipods (e.g., *Diporeia* spp.), isolating the food supply can be problematic and exact measurement of the AE difficult to determine (Harkey et al., 1994b). Thus, interpretation of measured AEs must be performed with care.

Clearly, as the log K_{ow} of a contaminant increases, there is a greater propensity to partition into lipid-rich tissues. However, when the contaminant must be transferred from a lipid phase (food) instead of an aqueous phase, then the relationship may be less straightforward, as described previously. Indeed, several studies indicated that there is an inverse relationship between AE and contaminant hydrophobicity (Bierman, 1990; Muir and Yarechewski, 1988). Thus, as the hydrophobicity of the contaminant increases, the lower the tendency to move from food lipid into the consumer's tissues.

9.6.3.2. Miscellaneous Factors Affecting Assimilation

The relationship between contaminant characteristics and AE can be modified by a variety of factors. The feeding rate and the amount of food ingested, for instance, can exert considerable influence on AE. When identical amounts of contaminants were fed to guppies, *Poecilia reticulata,* in two different volumes of food, the AE appeared to be lower in the case in which more food was used (Clark and Mackay, 1991). In reality, the AE declined because fecal egestion increased when a higher food volume was used. Thus, the thermodynamic tendency for the contaminant to move from food into the organism was not altered between exposures, but the processing of the food was quicker in the case of the high food volume, resulting in diminished contact time in the gut and an apparent reduction in absorption efficiency. Gut retention time is a key factor in determining absorption. Bruner et al. (1994b) found that AEs of several contaminants from sediment into zebra mussels were much lower than AE of the same contaminants from algae in part because the residence time of the sediment in the GIT was much lower.

Gut morphology can also be important in determining AE. For birds, the proximal part of the GIT appears to absorb more contaminants (Serafin, 1984). This may indicate a reduction in absorption efficiency in the distal portions of the GIT. Because the fugacity of the contaminant may be highest when the greatest digestion and removal of lipid has occurred in distal portion of the GIT, AE may decrease as a function of intestinal length.

In short, AE is known to be critical in assessing trophic transfer. Organism lipid levels are clearly important in determining AE. However, a variety of other factors that are not related to the adiposity of the organism is also influential. These interacting factors may obscure the relationship between AE and lipids.

9.7. Biomagnification and Organism Lipids

9.7.1. Is Biomagnification Real?

The issue of biomagnification has been a thorny one for years and has always revolved around lipid levels of each food chain element. An early report of biomagnification was the description of DDT levels in a Lake Michigan food chain (Harrison et al., 1970). In a simple food chain consisting of sediment, amphipods, fish, and herring gulls, they detected DDT concentrations of 14, 410, 3,000–6,000, and 99,000 ppb, respectively. The increase in DDT concentration with each trophic level was attributed to the fact that biomass conversion at each trophic link was <50% whereas DDT transfer was close to 80%. The concentration of DDT, thus, increased with each successive food chain link. Additional concentrating mechanisms were later identified. These included resistance to metabolism, high lipid solubility, and increasing lipid levels with each trophic link (Bierman, 1990).

Early reports of biomagnification were contested as a greater mechanistic understanding of the processes involved in accumulation evolved. In particular, the

finding that the contaminant uptake directly from water was by far the fastest route of accumulation, and that contaminant levels in top trophic levels could often be produced by bioconcentration alone (LeBlanc, 1995) caused doubt. In addition, failure to find biomagnification occurring in all ecosystems or in all components of a food chain was problematic. Highly hydrophobic contaminants such as PAHs, for instance, do not biomagnify (Burns and Teal, 1979) primarily as a result of biotransformation. Burrows and Whitton (1983) found that contaminant loads were higher in mayflies than in the predators that ate them. In many cases, contaminant loads varied randomly between trophic levels without discernible patterns (Biddinger and Gloss, 1984; Kay, 1984; Macek et al., 1979). In the absence of a mechanism to account for biomagnification, there was only inconsistent evidence to support its validity.

In a recent review of hundreds of studies on biomagnification, Suedel et al. (1994) concluded that, although the occurrence of biomagnification is much more limited than previously suggested, it is nonetheless a reality in some systems. Biomagnification appears to be limited to a small but very important group of highly lipophilic (log K_{ow} >5), nonmetabolized contaminants that include DDT, DDE, PCBs, toxaphene, and organic forms of mercury and arsenic. Biomagnification is precluded by, among other things, susceptibility to metabolism and high elimination rates and can be enhanced when contaminant elimination rates are slow compared with the energetics of the organism.

9.7.2. A Lipid-Based Model for Biomagnification

Mechanistically, biomagnification appears to consist of a continuation of the same process that leads to trophic transfer of contaminants. That is, as contaminated food is digested in the gut, lipids are absorbed and the food volume decreases. The fugacity of the contaminant in the food, thus, increases above the fugacity of the chemical in the organism and provides the force necessary to drive the contaminant across the gut by diffusion. The process can be increased by digestion of nonlipids (e.g., proteins and carbohydrates) because that process increases the surface area from which sorbed contaminants can be released (DiPinto et al., 1993). In addition, it is possible that the compositional changes of partially digested lipids in the gut will lead to a reduction in fugacity capacity (and increase in fugacity) without a reduction in food volume (LeBlanc 1995; Gobas et al., 1993a).

Although the currently accepted models for both trophic transfer and biomagnification in aquatic systems invoke passive diffusion as the force that transports contaminants across the GIT into the organism, models derived from terrestrial mammals have highlighted the possibility that contaminants can also be co-assimilated with dietary lipids. In co-assimilation, contaminants move across the GIT in association with lipids that are being absorbed; the lipids become the vehicle for contaminant transport. In the second model, the contaminant does not move by diffusion, because the chemical concentration and fugacity in the organism are similar or higher than levels in the food, but rather via an active process

that uses lipoprotein carriers. If the passive diffusion model is true, in the strictest sense, then the mode of transport is diffusion created by a fugacity gradient and the site of magnification is the GIT (Gobas et al., 1993b). If, however, the second model is true, then the mode of accumulation is lipid co-assimilation and the site of magnification is in the organism's (or predator's) tissues.

Gobas et al. (1993b) evaluated these two models experimentally in goldfish (*Carassius auratus*) by using chlorinated benzenes and PCBs. In this experiment, groups of goldfish were fed identical concentrations of contaminant in food, with lipid levels ranging from 0 to 13.5%. If the contaminants were transferred from food to fish strictly via lipid co-assimilation, then contaminant uptake should be very low in a diet deficient in lipid because lipids are the primary transport vehicle for the contaminants. By contrast, contaminant uptake in high-lipid diets should be significantly greater than from low-lipid diets if lipid co-assimilation predominates. If passive diffusion is responsible for contaminant transfer, then a dramatic change in dietary lipid content should occasion only a small alteration in the amount of contaminant taken up, assuming that fecal egestion rates do not change between treatments.

The results of the experiment suggested that although diffusion resulting from increased fugacity caused by digestion was the main force driving accumulation, lipid co-assimilation also played a role depending on the hydrophobicity of the contaminant. For the lower log K_{ow} contaminants, there was no difference in uptake efficiencies from low- versus high-lipid diets. That is, diffusion appeared to be the driving force behind accumulation, and magnification was taking place in the GIT. However, for hexa-, octa-, and decachlorobiphenyl, the uptake efficiency was significantly higher from low-fat food than from high-fat food due to greater digestibility (Gobas et al., 1993b). This indicated that the amount of transfer was tied to dietary lipid levels as suggested by the lipid co-assimilation model, albeit in the direction opposite that which was initially predicted. This was attributed to the higher digestibility of the low-lipid food. Still, the fact that contaminant uptake was associated with dietary lipid levels was viewed as evidence for the lipid co-assimilation model.

In fact, experimental results exist to support both models. For instance, a tenfold increase in dietary triglycerides had no effect on benzo[*a*]pyrene (BaP), 3-methylcholanthrene, or aminostilbene accumulation from food in rats (Laher et al., 1984; Kamp and Neumann, 1975). This lends credence to the passive diffusion model. Evidence in support of the lipid co-assimilation model has also been adduced, particularly for extremely hydrophobic contaminants such as BaP (Vetter et al., 1985; Rees et al., 1971).

Given the contradictory experimental results, Gobas et al. (1993b) proposed a model that includes aspects of both the diffusion and lipid co-assimilation mechanisms. In this view, contaminants remain associated with dietary lipids as they move from food into contact with intestinal mucosa. This association is particularly significant in the case of extremely hydrophobic contaminants for which disassociation into the aqueous milieu of the gut is thermodynamically and kinetically unfavorable. Thus, attaching to lipid material during the transport avoids

dissolution in an aqueous phase. Lipids are then digested and absorbed across the gut. The concentration and fugacity of the contaminants remaining in the gut then increases, providing the diffusive force needed to transfer the contaminants, as single molecules, into the organism. Thereafter, the contaminants may become reassociated with the products of lipid digestion, particularly triglycerides or lipoproteins, in which form they may circulate in the blood prior to deposition and storage in adipose tissues. Although both diffusion and lipid co-assimilation are components of the revised model, diffusion is identified as the rate-limiting step in the transfer process. As a consequence, the actual magnification of the contaminant residue level is seen as taking place in the GIT, not in the consumer's tissues (Gobas et al., 1993b).

9.7.3. Current Issues in Biomagnification and Relationship to Lipids

Despite the availability of a detailed mechanism to explain biomagnification and an abundance of residue data demonstrating biomagnification for a limited group of contaminants with fairly specific physicochemical requirements, the reality of biomagnification continues to be questioned. LeBlanc (1995) has suggested that much of what is misinterpreted as biomagnification is, in fact, simply bioconcentration. In support of this hypothesis, LeBlanc notes, in his limited survey, that lipid levels tend to increase with each trophic levels on a wet weight basis, lipids in phytoplankton average 0.5% ($n = 1$); in invertebrates, lipids average 1.8% ($n = 8$); and in fish, lipids average 5.4% ($n = 10$) (LeBlanc, 1995). Thus, contaminant loads would be expected to be higher in each successive trophic level due simply to passive uptake from water. Additionally, LeBlanc (1995) argues that the composition of lipid changes between trophic levels. If these compositional changes cause the fugacity capacity of lipids at higher trophic levels to increase, then even an increase in lipid levels would not be required to cause an increase in bioconcentration (or bioaccumulation). Finally, LeBlanc (1995) argues that differences in lipid elimination mechanisms between trophic levels could account for elevated contaminant loads in top trophic levels. That is, animal size tends to increase with trophic level. In addition, elimination of contaminants from lipid storage (and subsequent excretion from the body) requires that the contaminant be removed from lipid storage and transferred to an excretory organ, most likely the gill (respiratory organ) or liver (or functional equivalent). Liver cells have specific membrane sites across which contaminants must pass, and these elimination sites constitute a small fraction of the total organ surface area. As animal size increases, the relative abundance of storage-to-elimination sites rises. Also, as organism size increases, the relative size of the respiratory organ decreases. As a result, excretion via passive diffusion either via a liver function or through the respiratory system is much lower in large animals than in smaller animals at lower trophic levels. Similar findings of limited biomagnification were presented for freshwater benthic organisms (Bierman, 1990).

Although the arguments against biomagnification cited above are compelling, there are some situations in which accumulation of a contaminant in an organism above residues found in its food cannot be explained in any way other than biomagnification. For instance, Rasmussen et al. (1990) compared PCB concentrations in a single predatory fish species, lake trout (*Salvelinus namaycush*) from more than 80 different sites throughout Ontario. Despite the fact that the lake trout in all sites experienced similar environmental exposure levels to PCBs, the actual concentrations of PCBs in lake trout varied from 15 to 10,000 ppb. The between-lake variation in PCB content in lake trout was attributed to two factors: length of the food chain and increase in lipid levels as a function of food chain length. Each trophic link in the food chain resulted in a magnification of PCB concentrations by a factor of approximately 3–5. Thus, when the length of the food chain was relatively short, the lake trout were much less contaminated than when the food chain was longer. Lipid levels also increased with food chain length. However, lipid levels increased by a factor of 1.5 with each trophic link and cannot, therefore, account for the magnification of PCBs at each step. In other words, biomagnification of PCB residues from food must be occurring (Rowan and Rasmussen, 1992). A trophic position model for lake trout has been developed for both PCB and mercury biomagnification (Vanderzanden and Rasmussen, 1996). Similarly, biomagnification was found to account for high K_{ow} PCB congeners in white bass (*Morone chysops*) in the Lake Erie food web based on a fugacity analysis (Russell et al., 1995) and in fresh water, Lake Nieuwe Meer, in The Netherlands (van der Oost et al., 1988). Further, the presumed increase in total lipid content with each trophic step does not always occur while biomagnification is observed (Broman et al., 1992), thus refuting the arguments that it is increases in lipid content driving the presumed biomagnification put forth by LeBlanc (1995). In situ biomagnification has been difficult to demonstrate, in part because of the difficulty in placing organisms at appropriate levels within a food chain. However, with the use of stable isotope analysis, the biomagnification of polychlorinated dibenzodioxins and polychlorinated dibenzofurans was clearly demonstrated for a Baltic Sea food chain (Broman et al., 1992).

In short, the relative contributions of contaminated food versus contaminated media may vary between ecosystems or even between organisms within an ecosystem. However, residue data suggest that biomagnification of specific contaminants occurs in nature. This, combined with knowledge of a mechanism for understanding of the process of biomagnification and data that show magnification of residues from food when other avenues of contaminant exposure are precluded, provides strong support for the validity of biomagnification.

9.8. Lipids and Transgenerational Transfer of Contaminants

Loss of stored contaminant residues in female animals through reproduction is now understood as a key elimination mechanism, particularly in long-lived species (Sijm et al., 1992). For instance, contaminant residues in adult birds may be

passed to embryos via the egg (Burger and Gochfeld, 1993; Jarman et al., 1993; Heinz et al., 1989). In some cases, the concentrations of contaminants in eggs exceed the concentrations found in parental tissues (Heinz, 1993; Tillitt et al., 1992). Similar results have been obtained for reptiles (e.g., snapping turtles, in which lipid storage of contaminants in adult adipose tissue often protects the adult turtle from overt symptoms of poisoning). However, liberal transfer of maternal residues to eggs has been documented several times (Loganathan et al., 1995; Bishop et al., 1994; Struger et al., 1993). Physiological effects in contaminated embryos of both birds and reptiles such as depressions in the titers of key hormones (e.g., estradiol or enzymes) (Chen et al., 1994; Trust et al., 1994), gross structural abnormalities (Hansen, 1994), embryo death, and complete reproductive failure (van den Berg et al., 1994; Bishop et al., 1991) have been attributed to transgenerational transfer of contaminant loads.

Although reproductive processes are infrequently studied with contaminant fate as a focus, it is clear from what is known of reproductive physiology that lipid metabolism is likely to play a key role in transgenerational contaminant transfer. In birds, reptiles, amphibians, and fish, developing oocytes take up large amounts of maternally derived vitellogenin, which consists of about 20% lipid, to serve as an energy source during subsequent development. In addition, lipids are also taken up directly from maternal stores or synthesized de novo by the embryo from maternal extrahepatic lipid (Mommen and Walsh, 1988). The development of the embryo can be reasonably viewed as taking place in a lipid-rich environment. If the maternal lipids are contaminated, then transfer of the contaminants to the embryo could easily take place following the normal pathways of lipid deposition in the embryo during reproduction. Further, because the lipid content of the embryo is high, relative to maternal tissues, the increase in lipid content will increase the fugacity capacity of the embryo, decrease its fugacity (for a given concentration, increasing Z decreases f), and increase the tendency of the contaminant to move into the embryo. That is, a mechanism for explaining observed "reproductive magnification" of residues in embryonic tissues exists and relates directly to lipid content.

The above scenario is largely unstudied but presents several testable hypotheses that could serve as the focus for additional research. For instance, maternal contaminant loads (lipid normalized) should decrease after a brood has been produced if maternal lipid reserves are the source of embryonic contamination. There is at least one report confirming this assertion in fish (Sijm et al., 1992), but data in other species are needed. In addition, the hypothesis suggests that residues in embryos should increase in proportion to the lipid content of the embryo. Monitoring those changes throughout larval development should be informative. In any event, the primacy of lipid levels in determining all aspects of contaminant uptake highlights the need to examine its role in reproduction because the biological impacts on reproduction appear to be detrimental.

9.9. Conclusions

Lipids are the dominant force in determining organic contaminant accumulation in aquatic organisms. Lipid normalization eliminates most variability between species in bioconcentration studies and is, thus, useful in making predictions about bioconcentration from physical parameters such as log K_{ow}. Normalizing contaminant loads to lipid levels is also helpful in cases in which the contaminant must leave a lipid compartment and negotiate an intermediate aqueous phase before final deposition in a second lipid compartment (e.g., bioaccumulation and trophic transfer). Evidence varies as to whether total lipids or the abundance of specific lipid subclasses are the most relevant referent, but it is clear that lipid normalization will significantly reduce variation for comparing contaminant loads between species. This is an area that should be studied further.

Studies of the relationships between lipids and contaminant accumulation have led to new insights on obviously important but seldom studied phenomena. The role of lipids, for instance, in producing membrane narcosis is pivotal, and its study may elucidate important details of the mechanism for narcosis and how toxins can affect lipid levels, resulting in alterations in energy (lipid) stores and perhaps membrane function. In addition, lipid metabolism appears to account for biomagnification in which contaminants appear to accumulate in predators against a concentration gradient. Finally, study of lipid mobilization and deposition in reproduction may help to define the ability of contaminants to move into filial generations during reproduction; it may also provide insight into the sorts of toxin-induced biological effects that can be expected in contaminated offspring. In short, our ability to predict the movement of organic contaminants in aquatic systems and to project the effects of that contamination depends on an understanding of the importance of lipids. Because these are the key elements of risk assessment, our ability to conduct accurate assessments may reasonably be seen to depend on our knowledge of lipids and their dynamics.

Acknowledgments. We thank Duane Gossiaux for his help in generating the graphics for Figure 9.1. This chapter is GLERL contribution 1024.

References

Arts, M.T.; Headley, J.V.; Peru, K.M. Persistence of herbicide residues in *Gammarus lacustris* (Crustacea: Amphipoda) in prairie wetlands. Environ. Toxicol. Chem. 15:481–488; 1996.

Arts, M.T.; Ferguson, M.E.; Glozier, N.E.; Robarts, R.D.; Donald, D.B. Spatial and temporal variability in lipid dynamics of common amphipods: assessing the potential for uptake of lipophilic contaminants. Ecotoxicology 4:91–113; 1995.

Axelman, J.; Broman, D.; Naf, C.; Pettersen, H. Compound dependence of the relationship log K_{ow} and log BCF_L. Environ. Sci. Pollut. Res. 2:33–36; 1995.

Banerjee, S.; Baughman, G.L. Bioconcentration factors and lipid solubility. Environ. Sci. Technol. 25:536–539; 1991.

Barron, M.G. Bioconcentration. Environ. Sci. Technol. 24:1612–1618; 1990.

Bickel, M.H. The role of adipose tissue in the distribution and storage of drugs. Prog. Drug Res. 28:273–303; 1994.

Biddinger, G.R.; Gloss, S.P. The importance of trophic transfer in the bioaccumulation of chemical contaminants in aquatic systems. Residue Rev. 91:103–145; 1984.

Bierman, V.J. Equilibrium partitioning and biomagnification of organic chemicals in benthic animals. Environ. Sci. Technol. 24:1407–1412; 1990.

Bishop, C.A.; Brown, G.P.; Brooks, R.J.; Lean, D.R.S.; Carey, J.H. Organochlorine contaminant concentrations and their relationship to the body size and clutch characteristics of the female common snapping turtle in Lake Ontario, Canada. Arch. Environ. Contam. Toxicol. 27:82–87; 1994.

Bishop, C.A.; Brooks, R.J.; Carey, J.H.; Ng, P.; Norstrom, R.J.; Lean, D.R.S. The case for cause–effect linkage between environmental contamination and development in eggs of the common snapping turtle from Ontario, Canada. J. Toxicol. Environ. Health 33:521–547; 1991.

Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 39:911–917; 1959.

Brannon J.M.; Price, C.B.; Reiley, F.J., Jr., Pennington, J.C.; McFarland, V.A. Effects of sediment organic carbon on distribution of radiolabeled fluoranthene and PCBs among sediment, interstitial water and biota. Bull. Environ. Contam. Toxicol. 51:873–880; 1993.

Broman, D.; Näf, C.; Rolff, C.; Zebühr, R.; Fry, B.; Hobbie, J. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polyclorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the northern Baltic. Environ. Toxicol. Chem. 11:331–345; 1992.

Bruger, J.; Gochfeld, M. Lead and cadmium accumulation in eggs and fledgling seabirds in the New York Bight. Environ. Toxicol. Chem. 12:261–267; 1993.

Bruggerman, W.A.; Martron, L.B.J.M.; Koolman, D.; Hutzinger, O. Accumulation and elimination kinetics of di-, tri-, and tetrachlorobiphenyls by goldfish after dietary and aqueous exposure. Chemosphere 10:811–815; 1981.

Bruner, K.A.; Fisher, S.W.; Landrum, P.F. The role of the zebra mussel, *Dreissena polymorpha,* in contaminant cycling. I. The effect of body size and lipid content on the bioconcentration of PCBs and PAHs. J. Great Lakes Res. 20:725–734; 1994a.

Bruner, K.A.; Fisher, S.W.; Landrum, P.F. The role of the zebra mussel, *Dreissena polymorpha,* in contaminant cycling. II. Contaminant accumulation from ingested algal and suspended sediment particles and contaminant trophic transfer from zebra mussel feces to the benthic invertebrate, *Gammarus fasciatus.* J. Great Lakes Res. 20:735–750; 1994b.

Burns, K.A.; Teal, J.M. The West Falmouth oil spill: hydrocarbons in the salt marsh ecosystem. Est. Coastal Mar. Sci. 8:349–360; 1979.

Burrows, I.G.; Whitton, B.A. Heavy metals in water, sediments and invertebrates from a metal contaminated river free of organic pollution. Hydrobiologia 106:263–273; 1983.

Chen, S.W.; Dzuik, P.J.; Francis, B.M. Effect of four environmental toxicants on plasma Ca and estradiol 17B and hepatic P450 in laying hens. Environ. Toxicol. Chem. 13:789–795; 1994.

Chiou, C.T.; Freed, V.H.; Schmedding, D.W.; Kohnert, R.L. Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Technol. 11:475–478; 1977.

Clark, K.E.; Mackay, D. Dietary uptake and biomagnification of four chlorinated hydrocarbons by guppies. Environ. Toxicol. Chem. 10:1205–1217; 1991.

Connell, D.W. Bioaccumulation behavior of persistent organic chemicals with aquatic organisms. Rev. Environ. Contam. Toxicol. 101:117–154; 1988.

Cravedi, J.P.; Tulliez, J. Metabolism of n-alkanes and their incorporation into lipids in rainbow trout. Environ. Res. 39:180–187; 1986.

de Boer, J. Chlorobiphenyls in bound and non-bound lipids of fishes: comparison of different extraction methods. Chemosphere 17:1803–1810; 1988.

DiPinto, L.M.; Coull, B.C.; Chandler, G.T. Lethal and sublethal effects of sediment-associated PCB Arochlor 1254 on a meiobenthic copepod. Environ. Toxicol. Chem. 12:1909–1918; 1993.

DiToro, D.M.; Zarba, C.S.; Hansen, D.J.; Berry, W.J.; Swartz, R.C.; Cowan, C.E.; Pavlou, S.P.; Allen, H.E.; Thomas, N.A.; Paquin, P.R. Technical basis for establishing sediment quality criteria for nonionic organic chemicals by using equilibrium partitioning. Environ. Toxicol. Chem. 12:1541–1583; 1991.

Ellis, G.S.; Huckins, J.N.; Rostad, C.E.; Schmitt, C.J.; Petty, J.D.; MacCarthy, P. Evaluation of lipid-containing semipermeable membrane devices for monitoring organochlorine contaminants in the Upper Mississippi River. Environ. Toxicol. Chem. 14:1875–1884; 1995.

Ewald, G. Role of lipids in the fate of organochlorine compounds in aquatic ecosystems. Doctoral dissertation, Department of Ecology, Lund University, Lund, Sweden; 1996.

Ewald, G.; Larsson, P. Partitioning of ^{14}C-labelled 2,2′, 4,4′-tetrachlorobiphenyl between water and fish lipids. Environ. Toxicol. Chem. 13:1577–1580; 1994.

Fisher, S.W.; Gossiaux, D.C.; Bruner, K.A.; Landrum, P.F. Investigations of the toxicokinetics of hydrophobic contaminants in the zebra mussel (*Dreissena polymorpha*). In: Nalepa, T.F.; Schloesser, D.W., eds. Zebra Mussels: Biology, Impacts and Control. Boca Raton, FL: CRC Press; 1993:p. 453–464.

Folch, J.; Lees, M.; Cloane Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509; 1957.

Gardner, W.S.; Landrum, P.F.; Chandler, J.F. Lipid-partitioning and disposition of benzo(a)pyrene and hexachlrorbiphenyl in Lake Michigan, *Pontoporeia hoyi*. Environ. Toxicol. Chem. 10:35–46; 1990.

Gardner, W.S.; Frez, W.A.; Cichocki, E.A.; Parrish, C.C. Micromethod for lipids in aquatic invertebrates. Limnol. Oceanogr. 30:1100–1105; 1985.

Geyer, H.J.; Scheunert, I.; Bruggeman, R.; Matthies, M.; Steinberg, C.E.W.; Zitko, V.; Kettrup, A.; Garrison, W. The relevance of aquatic organisms lipid content to the toxicity of lipophilic chemicals: toxicity of lindane to different fish species. Ecotoxicol. Environ. Safety 28:53–70; 1994.

Geyer, H.J.; Scheunert, I.; Rapp, K.; Gebefugi, I.; Steinberg, C.; Kettrup, A. The relevance of fat content in toxicity of lipophilic chemicals to terrestrial animals with special reference to dieldrin and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Ecotoxicol. Environ. Safety 26:45–60; 1993.

Gobas, F.A.P.C.; Mackay, D. Dynamics of hydrophobic organic chemical bioconcentration in fish. Environ. Toxicol. Chem. 6:495–504; 1987.

Gobas, F.A.P.C.; Zhang, X.; Wells, R. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environ. Sci. Technol. 27:2855–2864; 1993a.

Gobas, F.A.P.C.; McCorquodale, J.R.; Haffner, G.D. Intestinal absorption and biomagnification of organochlorines. Environ. Toxicol. Chem. 12:567–577; 1993b.

Gobas, F.A.P.C.; McNeil, E.J.; Lovett-Doust, L.; Haffner, G.D. Bioconcentration of chlori-

nated aromatic hydrocarbons in aquatic macrophytes. Environ. Sci. Technol. 25:924–929; 1991.

Gobas, F.A.P.C.; Clark, K.E.; Shiu, W.Y.; Mackay, D. Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish: role of bioavailability and fecal elimination. Environ. Toxicol. Chem. 8:231–247; 1989.

Håkansson, H.; Sudlin, P.; Andersson, T.; Brunström, B.; Dencker, L.; Engwall, M.; Ewald, G.; Gilek, M.; Holm, G.; Honkassalo, S.; Idestam-Almquist, J.; Jonsson, P.; Kautsky, N.; Lundburg, G.; Lund-Kvernheim, A.; Martinsen, K.; Norrgren, L.; Pesonen, M.; Rundgren, M.; Sålberg, M.; Tarkpea, M.; Wesén, C. In vivo and in vitro toxicity of fractionated fish lipids, with particular regard to their content of chlorinated organic compounds. Pharmacol. Toxicol. 69:344–345; 1991.

Hansen, L.G. Halogenated aromatic compounds. In: Hansen, L.G.; Shane, B.S., eds. Basic Environmental Toxicology. Ann Arbor, MI: CRC Press; 1994:p. 199–230.

Harkey, G.A.; Landrum, P.F.; Kaline, S.J. Comparison of whole sediment, elutriate, and porewater for use in assessing sediment-associated organic contaminants in bioaccumulation assays. Environ Toxicol. Chem. 13:1315–1329; 1994a.

Harkey, G.A.; Lydy, M.J.; Kukkonen, J.; Landrum, P.F. Feeding selectivity and assimilation of PAH and PCB in *Diporeia* spp. Environ. Toxicol. Chem. 13:1445–1455; 1994b.

Harrison, H.L.; Loucks, O.L.; Mitchell, J.W.; Parkhurst, D.F.; Tracy, C.R.; Watts D.G.; Yannacone, V.J., Jr. System studies of DDT transport. Science 170:503–508; 1970.

Hawker, D.W.; Connell, D.W. Bioconcentration of lipophilic compounds by some aquatic organisms. Ecotoxicol. Environ. Safety 11:184–197; 1986.

Heinz, G.H. Selenium accumulation and loss in mallard eggs. Environ. Toxicol. Chem. 12:775–778; 1993.

Heinz, G.H.; Hoffamn, D.J.; Gold, L.G. Impaired reproduction of mallards fed and organic form of selenium. J. Wildl. Manage. 53:418–428; 1989.

Huckins, J.N.; Tubergen, M.W.; Manuweera, G.K. Semipermeable membrane devices containing model lipid: a new approach to monitoring and estimating their bioconcentration potential. Chemosphere 20:533–552; 1990a.

Huckins, J.N.; Tubergen, M.W.; Lebo, J.A.; Gale, R.W.; Schwartz, T.R. Polymeric film dialysis in organic solvent media for cleanup of organic contaminants. J. Assoc. Off. Anal. Chem. 73:290–293; 1990b.

Hunn, J.B.; Allen, J.L. Movement of drugs across the gills of fishes. Annu. Rev. Pharmacol. 14:47–55; 1974.

Jarman, W.M.; Burns, S.A.; Chang, R.R.; Stephens, R.D.; Norstrom, R.J.; Simon, M.; Linthicum, J. Determination of PCDDs, PCDFs and PCBs in California peregrine falcons (Falco peregrinus) and their eggs. Environ. Toxicol. Chem. 12:105–114; 1993.

Kaiser, K.L.E.; Vladmanis, I. Apparent octanol/water partition coefficients of pentachlorophenol as a function of pH. Can. J. Chem. 60:2104–2106; 1982.

Kamp, J.D.; Neumann, H.G. Absorption of carcinogens into the thoracic duct lymph of the rat: aminostilbene derivatives and 3-methylchloanthrene. Xenobiotica 5:717–727; 1975.

Kay, S.H. Cadmium in food webs. Residue Rev. 96:13–43; 1984.

Kenega, E.E. Correlation of concentration factors of chemicals in aquatic and terrestrial organism with their physical and chemical properties. Environ. Sci. Technol. 14:553–556; 1980.

Kucklick, J.R.; Harvey, H.R.; Ostrom, P.H.; Ostrom, N.E.; Baker, J.E. Organochlorine dynamics in the pelagic food web of Lake Baikal. Environ. Toxicol. Chem. 15:1388–1400; 1996.

Kucklick, J.R.; Bidelman, T.F.; McConnell, L.L.; Walla, M.D.; Ivanov, G.P. Organochlorines in the water and biota of Lake Baikal, Siberia. Environ. Sci. Technol. 28:31–37; 1994.

Kukkonen, J.; Landrum, P.F. Distribution of organic carbon and organic xenobiotics among different particle-size fractions in sediments. Chemosphere 32:1063–1076; 1996.

Kukkonen, J.; Landrum, P.F. Effects of sediment-bound polydimethylsiloxane on the bioavailability and distribution of benzo(a)pyrene in lake sediment to Lumbriculus variegatus. Environ. Toxicol. Chem. 14:523–531; 1995.

Laher, J.M.; Rigler, M.W.; Vetter, R.D.; Barrowman, J.A.; Patton, J.S. Similar bioavailability and lymphatic transport of benzo(a)pyrene when administered to rats in different amounts of dietary fat. J. Lipid Res. 25:1337–1342; 1984.

Lake, J.L.; Rubenstein, N.I.; Lee, H., II; Lake, C.A.; Heltshe, J.; Pavignano, S. Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. Environ. Toxicol. Chem. 9:1095–1106; 1990.

Lal, B.; Singh, T.P. Impact of pesticides on lipid metabolism in the freshwater catfish, *Clarias batrachus,* during the vitellogenic phase of its annual reproductive cycle. Ecotoxicol. Environ. Safety 13:13–23; 1987.

Landrum, P.F. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi:* role of physiological and environmental variables. Aquat. Toxicol. 12:245–271; 1988.

Landrum, P.F.; Dupuis, W.S. Toxicity and toxicokinetics of pentachlorophenol and carbaryl to *Pontoporeia hoyi* and *Mysis* relicta. In: Landis, W.G.; Van der Schalie, W. H., eds. Aquatic Toxicology and Risk Assessment, 13th vol. ASTM STP 1096. Philadelphia: American Society for Testing and Materials; 1990:p. 278–289.

Landrum, P.F.; Faust, W.R. The role of sediment composition on the bioavailability of laboratory-dosed sediment-associated contaminants to the amphipod, *Diporeia* spp. Chem. Speciat. Bioavail. 6:85–92; 1994.

Landrum, P.F.; Faust, W.R. Effect of variation in sediment composition on the uptake rate coefficient for selected PCB and PAH congeners by the amphipod, *Diporeia* spp. In: Mayes, M.A.; Barron, M.G., eds. Aquatic Toxicology and Risk Assessment, vol. 14. ASTM STP 1124. Philadelphia: American Society for Testing and Materials; 1991:p. 263–279.

Landrum, P.F.; Robbins, J.A. Bioavailability of sediment associated contaminants: a review and simulation model. In: Baudo, R.; Giesy, J.P.; Muntau, H., eds. Sediments: Chemistry and Toxicity of In-Place Pollutants. Chelsea, MI: Lewis Publishers; 1990:p. 237–263.

Landrum, P.F.; Dupuis, W.S.; Kukkonen, J. Toxicity and toxicokinetics of sediment-associated pyrene in *Diporeia* spp.: examination of equilibrium partitioning theory and residue effects for assessing hazard. Environ. Toxicol. Chem. 13:1769–1780; 1994.

Landrum, P.F.; Lee, H.; Lydy, M.J. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ. Toxicol. Chem. 11:1709–1725; 1992.

Landrum, P.F.; Eadie, B.J.; Faust, W.R. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* spp. Environ. Toxicol. Chem. 10:35–46; 1991.

Lassiter, R.R.; Hallam, T.G. Survival of the fattest: Implications for acute effects of lipophilic chemicals on aquatic populations. Environ. Toxicol. Chem. 9:585–595; 1990.

LeBlanc, G.A. Trophic level differences in bioconcentration of chemicals: Implications in assessing environmental biomagnification. Environ. Sci. Technol. 29:154–160; 1995.

Lee, H., II. Models, muddles and mud: predicting bioaccumulation of sediment associated pollutants. In: Burton, G.A., ed. Sediment Toxicity Assessment. Ann Arbor, MI: Lewis Publishers; 1992:p. 73–94.

Leo, A.; Hansch, C.; Elkins, D. Partition coefficients and their uses. Chem. Rev. 71:525–616; 1971.

Lien, G.J.; McKim, J.M. Predicting branchial and cutaneous uptake of 2,5,2′,5′-^{14}tetrachlorobiphenyl in fathead minnows (*Pimephales promelas*) and Japanese medaka (*Oryzias latipes*): rate limiting factors. Aquat. Toxicol. 27:15–32; 1993.

Lien, G.J.; Nichols, J.W.; McKim, J.M.; Gallinat, C.A. Modeling the accumulation of three waterborne chlorinated ethanes in fathead minnows (*Pimephales promelas*): a physiologically based approach. Environ. Toxicol. Chem. 13:1195–1205; 1994.

Loganathan, B.; Kannan, K.; Watanabe, I.; Kawano, M.; Irvine, K.; Kumar, S.; Sikka, H. Isomer-specific determination and toxic evaluation of polychlorinated biphenyls and dioxins. Environ. Sci. Technol. 29:1832–1838; 1995.

Ma, L.; Taraschi, T.F.; Janes, N. Nuclear magnetic resonance partitioning studies of solute action in lipid membranes. Bull. Mag. Reson. 14:293–98; 1992.

McCarty, L.S.; Mackay, D. Enhancing ecotoxicological modeling and assessment. Environ. Sci. Technol. 27:1719–1728; 1993.

Macek, K.J.; Petrocelli, S.R.; Sleight, B.H., III. Consideration in assessing the potential for and significance of, biomagnification of chemical residues in aquatic foodchains. In: McFarland, V.A. Activity-based evaluation of potential bioaccumulation from sediments. In: Montgomery, R.L.; Leach, J.W., eds. Dredging and Dredged Material Disposal Proceedings of the Conference Dredging '84. New York: American Society of Civil Engineering; 1984:p. 461–466.

McFarland, V.A.; Clark, J.U. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. Environ. Health Perspect. 81:225–239; 1989.

Mackay, D. Multimedia Environmental Models: The Fugacity Approach. Chelsea, MI: Lewis Publishers; 1991.

Mackay, D. Correlation of bioconcentration factors. Environ. Sci. Technol. 16:274–278; 1982.

Marking L.L.; Kimerle, R. A., eds. Aquatic Toxicology. ASTM STP 667. Philadelphia: American Society for Testing and Materials; 1979:p. 251–268.

Mommen, T.P.; Walsh, P. J. Vitellogenesis and oocyte assembly. Vol. 11. In: Hoar, W.S.; Randall, D. J., eds. Fish Physiology. New York: Academic Press; 1988:p. 347–406.

Moriarty, F. Exposure and residues. In: Moriarty, F., ed., Organochlorine Insecticides: Persistent Organic Pollutants. New York: Academic Press; 1975:p. 29–72.

Muir, D.C.G.; Yarechewski, G.R.B. Dietary accumulation of four chlorinated dioxin congeners by rainbow trout and fathead minnows. Environ. Toxicol. Chem. 7:227–235; 1988.

Mullins, L.J. Some physical mechanisms in narcosis. Chem. Rev. 54:289–323; 1954.

Neely, W.B.; Branson, D.R.; Blau, G.E. Partition coefficient measure bioconcentration potential of organic chemicals in fish. Environ. Sci. Technol. 8:1113–1115; 1974.

Nichols, J.W.; McKim, J.M.; Lien, G.J.; Hoffman, A.D.; Bretelsen, S.L. Physiologically-based toxicokinetic modeling of three waterborne chloroethanes in rainbow trout (*Oncorhynchus mykiss*). Toxicol. Appl. Pharmacol. 110:374–389; 1991.

Nichols, J.W.; McKim, J.M.; Anderson, M.E.; Gargas, H.J.; Clewell, H.J., III; Erickson, R.J. A physiologically-based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish. Toxicol. Appl. Pharmacol. 106:433–447; 1990.

Opperhuizen, A.; Damen, H.W.J.; Asyee, G.M.; Van der Steen, J.M.D.; Hutzinger, O. Uptake and elimination by fish of polydimethylsiloxanes (silicones) after dietary and aqueous exposure. Toxicol. Environ. Chem. 13:265–285; 1987.

Opperhuizen, A.; Velde, E.W.; Gobas, F.A.P.C.; Liem, D.A.K.; Van der Steen, J.M.D.; Hutzinger, O. Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. Chemosphere 14:1871–1896; 1985.

Pawlisz, A.V.; Peters, R.H. A radioactive tracer technique for the study of lethal body burdens of narcotic organic chemicals in *Daphnia magna*. Environ. Sci. Technol. 27:2795–2800; 1993a.

Pawlisz, A.V.; Peters, R.H. A test of the equipotency of internal burdens of nine narcotic chemicals using *Daphnia magna*. Environ. Sci. Technol. 27:2801–2806; 1993b.

Prest, H.F.; Jarman, W.M.; Burns, S.A.; Weismüller, T.; Martin, M.; Huckins, J.N. Passive water sampling via semipermeable membrane devices (SPMDS) in concert with bivalves in the Sacramento/San Joaquin river delta. Chemosphere 25:1811–1823; 1992.

Randall, R.C.; Lee, H., II; Ozretich, R.J.; Lake, J.L.; Purell, R.J. Evaluation of selected lipid methods for normalizing pollutant bioaccumulation. Environ. Toxicol. Chem. 10:1431–1436; 1991.

Rasmussen, J.B.; Rowan, D.J.; Lean, D.R.S.; Carey, J. H. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish Aquat. Sci. 47:2030–2038; 1990.

Rees, D.E.; Mandelstam, P.; Lowry, J.Q.; Lipscomb, L.N. A study of the mechanism of intestinal absorption of benzo(a)pyrene. Biochim. Biophys. Acta 225:96–107; 1971.

Reynoldson, T.B. Interactions between sediment contaminants and benthic organisms. Hydrobiology 149:53–66; 1987.

Rowan, D.J.; Rasmussen, J.B. Why don't Great Lakes fish reflect environmental concentrations of organic contaminants?—An analysis of between-lake variability in the ecological partitioning of PCBs and DDT. J. Great Lakes Res. 18:724–741; 1992.

Rubenstein, N.I.; Gilliam, W.T.; Gregory, N.R. Dietary accumulation of PCBs from a contaminated sediment source by a demersal fish (*Leiostomus xanthurus*). Aquat. Toxicol. 5:331–342; 1984.

Russell, R.W.; Lazar, R.; Haffner, G.D. Biomagnification of organochlorines in Lake Erie white bass. Environ. Toxicol. Chem. 14:719–724; 1995.

Saito, S.; Tateno, C.; Tanoue, A.; Matsuda, T. Electron microscope autoradiographic examination of uptake behavior of lipophilic chemicals into fish gill. Ecotoxicol. Environ. Safety 19:184–191; 1990.

Schneider, R. Polychlorinated biphenyls (PCBs) in cod tissues from the western Baltic: significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill-breathing animals. Meeresforsch. 29:69–79; 1981.

Schultz, I.R.; Hayton, W.L. Body size and the toxicokinetics of trifluralin in rainbow trout. Toxicol. Appl. Pharmacol. 129:138–145; 1994.

Serafin, J.A. Avian species differences in intestinal absorption of xenobiotics (PCBs, Dieldrin, Hg^{2+}). Comp. Biochem. Physiol. 78:491–496; 1984.

Shaw, G.R.; Connell, D.W. Factors controlling bioaccumulation in food chains. In: Waid, J.S., ed. PCBs and the Environment. vol. I. Boca Raton, FL: CRC Press; 1986:p. 135–141.

Shaw, G.R.; Connell, D.W. Physicochemical properties controlling polychlorinated biphenyl (PCB) concentrations in aquatic organisms. Environ. Sci. Technol. 18:18–23; 1984.

Sijm, D.T.H.M.; Seinen, W.; Opperhuizen, A. Life-cycle biomagnification study in fish. Environ. Sci. Technol. 26:2162–2174; 1992.

Södergren, A. Solvent-filled dialysis membranes simulate uptake of pollutants by aquatic organisms. Environ. Sci. Technol. 21:855–863; 1987.

Södergren, A.; Okla, L. Simulation of interfacial mechanisms with dialysis membranes to study uptake and elimination of persistent pollutants in aquatic organisms. Verh. Int. Verein. Limnol. 23:1633–1638; 1988.

Stange, K.; Swackhamer, D.L. Factors affecting phytoplankton species-specific differences in accumulation of 40 polychlorinated biphenyls (PCBs). Environ. Toxicol. Chem. 13:1849–1860; 1994.

Stehly, G.R.; Hayton, W.L. Effect of pH on the accumulation kinetics of pentachlorophenol in goldfish. Arch. Environ. Contam. Toxicol. 19:464–470; 1990.

Struger, J.; Elliot, J.E.; Bishop, C.A.; Obbard, M.E.; Norstrom, R.J.; Weseloh, D.V.; Simon, M.; Ng, P. Environmental contaminants of the common snapping turtle from the Great Lakes-St. Lawrence River basin of Ontario, Canada (1981–1984). J. Great Lakes Res. 19:681–694; 1993.

Suedel, B.C.; Boraczek, J.A.; Peddicord, R.K.; Clifford, P.A.; Dillon, T.M. Trophic transfer and biomagnification potential of contaminants in aquatic ecosystems. Rev. Environ. Contam. Toxicol. 136:21–84; 1994.

Swackhamer, D.L.; Hites, R.A. Occurrence and bioaccumulation of organochlorine compounds in fishes from Siskiwit Lake, Isle Royal, Lake Superior. Environ. Sci. Technol. 22:543–548; 1988.

Swartz, R.C.; Lee, H., II. Biological processes affecting distribution of pollutants in marine sediments. Part I. Accumulation, trophic transfer, biodegradation and migration. In: Baker, R.A., ed. Contaminants and Sediments. Ann Arbor, MI: Ann Arbor Science Publishers; 1980:p. 534–563.

Thomann, R.V. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ. Sci. Technol. 23:699–707; 1989.

Thomann, R.V.; Connolly, J.P. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18:65–71; 1984.

Tillitt, D.E.; Ankley, G.T.; Giesy, J.P.; Ludwig, J.P.; Kurita-Matsuba, H.; Weseloh, D.V.; Ross, P.S.; Bishop, C.A.; Sileo, L.; Stromborg, K.L.; Larson, J.; Kubiak, T.J. Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes. Environ. Toxicol. Chem. 11:1281–1288; 1992.

Trust, K.A.; Fairbrother, A.; Hooper, M.J. Effects of 2,3,7,8-tetrachlorodibenz(a)anthracene on immune function and mixed function oxidase in the European starling. Environ. Toxicol. Chem. 13:821–830; 1994.

Tulasi, S.J.; Reddy, P.U.M.; Ramana Rao, J. V. Accumulation of lead and effects on total lipids and lipid-derivatives in the freshwater fish *Anabas testudineus* (Bloch). Ecotoxicol. Environ. Safety 23:33–38; 1992.

van den Berg, M.E.J.; Craane, B.L.H.J.; Sinnige, T.; van Mourik, S.; Dirksen, S.; Boudewijn, T.; van der Gaag, M.; Lutke-Schipholt, I.J.; Spenkelink, B.; Brouwer, A. Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. Environ. Toxicol. Chem. 13:803–816; 1994.

van den Heuvel, M.R.; McCarty, L.S.; Lanno, R.P.; Hickie, B.E.; Dixon, D.G. Effect of total body lipid on the toxicity and toxicokinetics of pentachlorophenol in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 20:235–252; 1991.

van der Oost, R.; Heida, H.; Opperhuizen, A. Polychlorinated biphenyl congeners in sediments, plankton, mollusks, crustaceans, and eel in a freshwater lake: Implications of using reference chemicals and indicator organisms in bioaccumulation studies. Arch. Environ. Contam. Toxicol. 17:721–729; 1988.

Vanderzaden, M.J.; Rasmussen, J.B. A trophic position model of pelagic food webs—

impact on contaminant bioaccumulation in lake trout. Ecol. Monogr. 66:451–477; 1996.

van Wezel, A.P.; Opperhuizen, A. Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms and membrane burdens. Crit. Rev. Toxicol. 25:255–279; 1995.

van Wezel, A.P.; de Vries, D. A.M.; Kostense, S.; Sijm, D.T. H.M.; Opperhuizen, A. Intraspecies variation in lethal body burdens of narcotic compounds. Aquatic Toxicol. 33:325–342; 1995.

Veith, G.D.; Macek, K.J.; Petrocelli, S.R.; Carroll, J. An evaluation of using partition coefficients and water solubility to estimates bioconcentration factors for organic chemicals in fish. In: Eaton, J.G.; Parrish, P.R.; Hendricks, A. C., eds. Aquatic Toxicolgy. ASTM STP 707. American Society for Testing and Materials; 1980:p. 116–129.

Veith, G.D.; DeFoe, D.L.; Bergstedt, B.V. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Bd. Can. 36:1040–1048; 1979.

Vetter, R.D.; Carey, M.C.; Patton, J.S. Co-assimilation of dietary fat and benzo(a)pyrene in the small intestine; an absorption model using killifish. J. Lipid Res. 26:428–434; 1985.

Walter, A.; Gutknecht, J. Permeability of small nonelectrolytes through lipid bilayer membranes. J. Membrane Biol. 90:207–217; 1986.

Westhall, J.C. Influence of pH and ionic strength on the aqueous-nonaqueous distribution of chlorinated phenols. Environ. Sci. Technol. 19:193–198; 1985.

Zitko, V. Metabolism and distribution by aquatic animals. In: Hutzinger, O., ed. The Handbook of Environmental Chemistry. vol. 2, part A. Berlin: Springer; 1980:p. 221–229.