American Petroleum Institute

STEP Strategies for Today's Environmental Partnership

BIOACCUMULATION:

HOW CHEMICALS MOVE FROM THE WATER INTO FISH AND OTHER AQUATIC ORGANISMS

Health and Environmental Sciences Department
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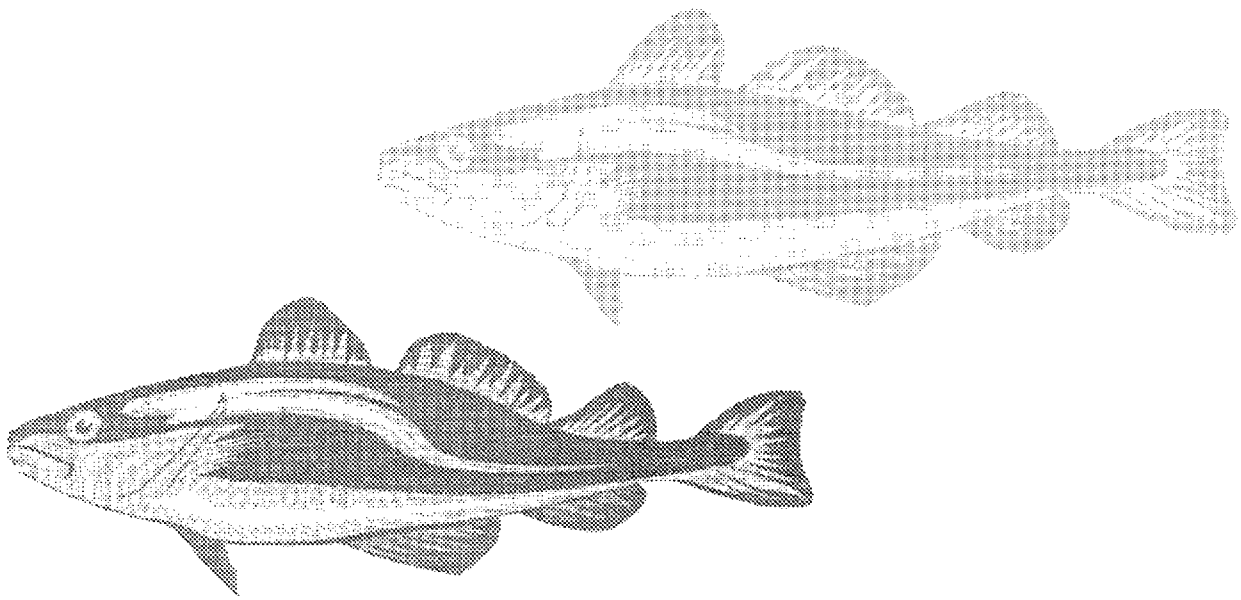

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Bioaccumulation:
How Chemicals Move from the Water into Fish and Other Aquatic Organisms

Health and Environmental Sciences Department

API PUBLICATION NUMBER 4656

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ABSTRACT

The purpose of this work is to provide an intermediate-level primer on *why* and *how* chemicals are accumulated by aquatic organisms (bioaccumulation). This is an important issue because of the potential effects of bioaccumulated chemicals on fish, wildlife, and ultimately, on humans. The chemicals emphasized in this primer are the polycyclic aromatic hydrocarbons (PAHs) and in particular the sixteen priority pollutant PAHs (selected by the U.S. EPA). Aquatic organisms are emphasized, but much of this information applies to terrestrial organisms as well. Key factors governing bioaccumulation are described to facilitate an understanding of this complex phenomenon. The factors include those related to the properties of the contaminant, the characteristics of the exposure media (environment), the organisms, and the supporting food chains. Several draft EPA approaches for assessing bioaccumulative substances are critically reviewed. Also, other potential assessment options such as use of transplanted sentinel organisms and lipid-containing semipermeable membrane devices (SPMDs) are examined. This report shows that although considerable information exists on the bioaccumulation phenomenon, there is a critical need for improved methods of assessing the presence of bioaccumulative chemicals. Of the bioaccumulation assessment methods examined for PAHs, the use of SPMDs offers the most potential. Finally, this work suggests that the likelihood for PAHs to have large bioaccumulation factors is relatively low.

EXECUTIVE SUMMARY

Chemicals that have a propensity to concentrate in aquatic life to levels higher than those found in the ambient environment (water) are characterized as bioconcentratable or bioaccumulative substances. Bioaccumulation includes the uptake of chemicals from both water and diet whereas bioconcentration represents uptake from water alone. Even though many contaminants are often present in the environment only at trace [less than a part-per-million (ppm)] or ultra trace [less than a part-per-trillion (ppt)] levels, they can accumulate to toxicologically significant levels in the fatty tissues of exposed organisms. The driving force behind this bioaccumulation phenomenon is the propensity of many chemicals to have much higher solubilities in organism lipid (fat) than in the ambient water. Another way to view the bioaccumulation phenomenon is that lipid-loving (lipophilic) contaminants have much lower escaping tendencies (fugacities) from fatty tissues than from water.

Many industrial processes generate wastes with low levels of chemicals that may bioaccumulate. Because of the potential for trace concentrations of these chemicals to adversely affect ecosystems and human health, the U.S. Environmental Protection Agency (EPA) is in the process of drafting methods for the assessment of bioaccumulative substances in industrial effluents. To ensure that bioaccumulative chemicals are not present at unacceptable levels in industrial outfalls or effluents, industry needs to be knowledgeable on the physical-chemical properties that are characteristic of these types of chemicals, how they interact with the environment, and the potential approaches available for their assessment.

This primer on bioaccumulation, prepared under the direction of API's Biomonitoring Task Force, is written for personnel with technical or scientific training, but without specific expertise in the subject matter. Although bioaccumulation is a complex subject, the authors have attempted to explain key aspects without using highly technical

treatment of details. A glossary is included to provide the reader with definitions of *important* terms related to bioaccumulation. Because several terms are defined only in the glossary and some variability exists in their use in the literature, reading the glossary is recommended. For example, in some literature bioaccumulation and bioconcentration are used interchangeably.

Several classes of organic chemicals have the potential to bioconcentrate or bioaccumulate. Also, certain organometal complexes may bioconcentrate. The focus of this work is on the polycyclic aromatic hydrocarbons (PAHs), and in particular, the EPA "priority pollutant" PAHs. Energy production and use appear to be the primary sources of low levels of PAHs in the environment. The properties of a number of chlorinated hydrocarbons are also examined for comparative purposes. The organisms emphasized are aquatic but much of the information presented also applies to terrestrial life.

The body of this report is divided into two parts: factors affecting bioaccumulation and approaches for assessing bioaccumulative substances. The reader should be aware that the classification of these factors into separate subsections is operational and does not necessarily imply that they are independent of each other.

FACTORS AFFECTING BIOACCUMULATION

Four types of variables affect bioaccumulation—physical-chemical properties of the contaminant molecules, environmental conditions, characteristics of the exposed organism, and the organism's food chain. These factors may act in concert or in opposition resulting in a range of bioaccumulation potentials.

Chemical Related Factors

Physical-chemical properties of the contaminant molecule play a central role in the bioaccumulation process. Features of chemicals that confer the tendency to

bioaccumulate include (1) lipophilicity or fat loving, which is directly related to the magnitude of a chemical's solubility in octanol, and characterized by the magnitude of the octanol-water partition coefficient (K_{ow}); (2) low water solubility or hydrophobicity due to the lack of polar functional groups; and (3) structural stability resulting in environmental persistence (years instead of days). Finally, chemicals of moderate molecular weight and size (i.e., molecular weight of about 350 and molecular breadth of less than 10 Angstroms), and lacking ionizable functional groups have a greater tendency to bioaccumulate.

Environmental Related Factors

As suggested earlier, the environmental presence of chemicals that meet most of the aforementioned criteria does not always lead to high degrees of bioaccumulation. This attenuation of bioaccumulation is often due to low residue bioavailability. For bioaccumulation to occur, a molecule must make contact with a biomembrane and move through the membrane to lipid-rich storage sites. The amount of chemical making contact with an organism's absorbing membranes is dependent not only on its environmental concentration in the bulk water phase (includes particulates), but also on the fractional amount that is available for uptake (i.e., the bioavailable fraction). This bioavailable fraction usually corresponds with the fraction of chemical that is truly dissolved in water.

Lipophilic or bioaccumulative chemicals also have high affinities for particulate organic carbon in suspended and bed sediments because the organic carbon associated with sediments has some of the same chemical characteristics as lipid. Most of the mass of a highly lipophilic contaminant in an aquatic system is usually not dissolved in the water but rather is sorbed on particulate organic carbon. The desorptive release of a lipophilic residue from sediment organic carbon can be very slow, thus significantly reducing the amount of chemical available for bioaccumulation. However, the slow release of sediment-sorbed contaminants in areas where contaminant inputs have declined is often the major source of trace levels of bioconcentratable compounds.

Another factor that greatly affects the potential of a compound to bioaccumulate is its environmental stability or persistence. The effects of environmental degradation processes (e.g., hydrolysis, photolysis and microbial degradation) on contaminant molecules typically result in more hydrophilic (water-loving) or polar products, which have lower bioaccumulation potentials than did the parent compounds. Some exceptions to this outcome do exist, especially under conditions of low oxygen. However, the overall effect of these degradation processes is to reduce parent compound concentrations and organism exposure time, thereby decreasing the amounts of residues bioaccumulated.

Organism Related Factors

Lipophilic contaminants are accumulated by aquatic organisms from water via respiration, and from ingested food or sediments. Bioconcentration (uptake from water) is generally viewed as the predominant route of uptake for most chemicals (including most PAHs) by aquatic organisms. Because liters of water per day are ventilated across the gill membranes of fish, the gill is generally the principal point of contaminant entry into an aquatic organism. The assimilation efficiencies of a variety of lipophilic compounds by this route range from about 20 to 90% of the contaminant residues present in ventilated water.

Diet is more likely to be the major route of uptake when chemicals are persistent and have high K_{ow} (i.e., greater than 10^5). This is especially true for the top predators (aquatic and terrestrial) of a food chain. The assimilation efficiency of lipophilic chemicals across the gut is dependent on the quality of the ingested materials. If ingested materials are largely nondigestable, such as most natural sediment organic carbon, then the likelihood of gastrointestinal uptake is diminished. Gut assimilation efficiencies for a series of lipophilic chemicals, from high quality fish food (e.g., animal or plant tissues), have been shown to range from about 50 to 85%. Note that lipid content of the consumer organism has little or no effect on dietary and respiratory uptake rates of chemicals but it does affect the ultimate capacity of an organism to accumulate a chemical.

Bioaccumulation occurs only if the rate of a chemical's uptake exceeds the rate of its elimination. In aquatic organisms, depuration of many lipophilic chemicals occurs passively across the gills. This route of elimination appears to be most important for nonpolar compounds that are not biotransformed. The rates of elimination for these compounds are generally inversely related to their K_{ow}.

In many organisms (especially mammals, birds, and aquatic vertebrates), both the enzyme system known as the cytochrome mixed-function monooxygenase (MFO) system, and the aryl hydrocarbon hydroxylase system are responsible for the biotransformation of a variety of lipophilic compounds, especially the PAHs. Remember that biotransformation products are typically more hydrophilic and have much more rapid elimination rates then their parent compounds. In fish, birds, and mammals, most MFO activity is localized in the liver and the route of elimination of the more hydrophilic metabolites is by the bile. Although the MFO system effectively detoxifies and reduces the bioaccumulation of many contaminants, certain PAHs and alkanes can be transformed to intermediates that are more toxic (including carcinogenic) than the parent compounds.

The ability to eliminate accumulated PAH residues by all processes varies among species according to the following general trend: mammals > fishes > crustaceans > bivalve molluscs. The typically low elimination rates of PAH and other contaminant residues by bivalves, which leads to high bioaccumulation, accounts in part for their popularity as sentinel or biomonitoring organisms.

Food Chain Related Factors

Biomagnification is the increase in the bioaccumulation factors (BAFs) of certain chemicals in organisms occupying sequentially higher trophic positions in a food chain. This phenomenon occurs because of the following sequence of events. As lipids of contaminated prey are digested in the gut of predators, the capacity of the digestate

(due to its increased polarity) to retain nonmetabolized lipophilic contaminants is reduced, resulting in the net transfer of these chemicals to the predator's lipid-rich tissues. Then, assuming that the predator continues to consume numerous prey, the rates of uptake by the diet can exceed the rate of elimination, resulting in contaminant concentrations higher than those that would be found in the predator's fatty tissues at equilibrium. If this animal is, in turn, consumed by a predator of higher trophic level, a further magnification in residue concentrations can occur. In cases where the predators are fish-eating birds and mammals having high consumption rates of contaminated fatty prey and limited elimination pathways, biomagnification can result in residue concentrations that are 100-fold higher than the equilibrium values.

BIOACCUMULATION ASSESSMENT

Three approaches are being considered by EPA to assess the presence of bioconcentratable or bioaccumulative substances (not covered by water quality criteria) in surface waters and effluents. These approaches are the tissue residue measurement option, effluent measurement option, and sediment assessment option. In this primer, only the salient features of these options are covered and an evaluation of each is provided. Also, the use of transplanted bivalve molluscs and lipid-containing semipermeable membrane devices (SPMDs) is examined as alternative options for the determination of bioaccumulative chemicals.

Tissue Residue Option

The tissue residue approach involves measuring the concentrations of contaminants in tissue samples of indigenous organisms from receiving water sites and comparing these values with those in similar organisms collected from relatively uncontaminated control sites. The tissue residue measurement approach is environmentally realistic. However, the same or similar species may not be collectible at the test and reference/control sites, there are potential differences in the residence time of the test organisms at the sites, there are differences in the abilities of different organisms to

eliminate contaminants, and costs of tissue collection and analysis are high. These factors limit the certainty and practicality of the tissue residue option.

Effluent Option

This approach involves the collection of samples of effluent water, the extraction of the organic chemicals from the water, and the separation and analysis of the bioconcentratable chemicals in the extracts. The effluent separation procedure is designed to sort the results of the initial screening analysis in order to determine which of the contaminants pose a hazard. The bioconcentration factors (BCFs) of those compounds identified in the effluent that appear to pose a hazard are estimated from log K_{ow}-log BCF relationships or obtained from measured values of BCFs in the literature that followed accepted standards for fishes and saltwater bivalve molluscs.

The effluent assessment option does not allow detection of all bioconcentratable chemicals that may be present in aquatic organisms. The approach is fairly robust for chlorinated hydrocarbons but may fail to detect many PAHs because of the likelihood of acid-mediated PAH degradation during sample cleanup. Other limitations include analytical interferences from the hydrocarbons often present in refinery effluents, lack of sensitivity of the analytical method, and the probable lack of reliably measured BCFs and/or BAFs for detected bioaccumulative substances.

Sediment Assessment Option

In some receiving waters, sediments may be a significant source of bioaccumulative chemicals. Since sediments can accumulate pollutants over relatively long periods of time and can be preferential sorption sites, contaminant residues are generally present at greater concentrations in sediments than in the overlying water. This characteristic can facilitate detection of contaminants that are only present in an effluent or other source at very low concentrations or that are only released periodically. Because of the sample cleanup procedures used by EPA, this option is useful only for acid-stable

compounds. Other limitations include potential interferences in sediment samples that can reduce analytical sensitivity and preclude analysis, and lack of reliable organic carbon partition coefficient (K_{oc}) and BCF values for many bioaccumulative substances. However, unlike the tissue residue option, it should be possible to detect a broader spectrum of potentially bioconcentratable chemicals.

Evaluation of EPA Assessment Options

Recently, fifteen laboratories participated in a round-robin study on the three options proposed by EPA for assessment of bioconcentratable contaminants (not covered by water quality criteria) in effluents and receiving waters. The data obtained by five participating laboratories have been critically reviewed and are the subject of a peer-reviewed journal article (Wong *et al.,* 1997). In general the proposed multi-step procedures for each matrix were found to be complex, prone to loss of the chemicals of interest, and unnecessarily time-consuming.

Other potential options

Two additional approaches for the assessment of bioconcentratable chemicals are examined in this work. These are the use of transplanted sentinel organisms and semipermeable membrane devices (SPMDs). The use of transplanted organisms is essentially a modification of EPA's tissue residue option; the potential of selecting a sentinel species having minimal capability to metabolize analytes of interest and maximal probability to survive at the sample sites is a significant improvement. Unfortunately, sentinel organisms with very low contaminant backgrounds are often unavailable. Also, sentinel organisms are not exempt from a variety of factors (e.g., health, water quality, etc.) known to influence the bioaccumulation of contaminants by aquatic organisms. Finally, the cost of applying this modification of the tissue residue option can be even greater than any of EPA's other options.

Lipid-containing SPMDs represent a new technology, and are designed to mimic the bioconcentration of bioavailable organic contaminants by aquatic organisms without

many of the variables associated with the use of live animals. Conceptually, SPMDs represent a bridge between EPA's tissue residue and effluent options. The devices can be used to estimate the concentrations of bioavailable (dissolved residues; values which are directly comparable to water quality criteria) aqueous contaminants (effluent option) as well as the bioconcentration potentials of chemicals (tissue residue option). SPMDs function well in all environmental water qualities. Data on the SPMD sampling rates (needed for water concentration estimation) for the priority pollutant PAHs have been generated. Uniform devices with extremely low contaminant levels are commercially available. Also, the cost of an SPMD study is considerably less than any of the other options discussed herein. As is true of EPA's effluent and sediment options, SPMDs do not provide data on the dietary uptake of chemicals or on their potential to biomagnify up the food chain. However, the major route of PAH uptake by aquatic organisms appears to be via water. Overall, SPMDs show considerable promise as a new tool for the assessment of bioaccumulative substances, whether used in conjunction with other methods or as a stand-alone approach.

SUMMARY

In this work, certain structural features of chemicals that increase the probability of bioaccumulation in aquatic organisms are delineated. Also, several environmental variables are highlighted that can enhance or reduce the magnitude of a chemical's bioaccumulation in organisms. The roles of organism physiology and diet are shown to be key factors in the bioaccumulation of some chemicals. In particular, differences in the abilities of aquatic species to eliminate the same chemical are shown to greatly affect the magnitude of bioaccumulation. Current and potential approaches for assessing bioaccumulative substances in water are reviewed and evaluated.

For those interested in additional information on this subject, references (Connell, 1990) and (Burkhard *et al.,* 1991) are key sources. In addition, Volume 2 of the "Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Chemicals"

(Mackay *et al.*, 1992) contains an excellent compilation of data directly and indirectly related to the BAFs of PAHs and other aromatic hydrocarbons.

Section 1
INTRODUCTION

The lipids or fats of fish and wildlife, and humans as well, act as a natural sink for certain types of chemicals. Chemicals that have a propensity to accumulate in aquatic organisms to levels higher than those found in water are called bioconcentratable or bioaccumulatable. Although bioconcentration and bioaccumulation are often used interchangeably in the literature, bioconcentration refers to chemical uptake from water or air, whereas bioaccumulation refers to chemical uptake from water or air and ingested food and/or sediment. Environmental contaminants that bioaccumulate are hydrophobic (water-hating) and lipophilic (lipid-loving) in nature. Most contaminants are transported across biological membranes by passive diffusion. The mechanism by which these contaminants concentrate in an organism's lipid can be expressed by a basic rule of thumb in chemistry, i.e., "like dissolves like." The level of a chemical in fatty tissues reflects the difference in its solubility in the lipid and the surrounding water, which is best described as liquid-liquid partitioning. The magnitude of a chemical's lipid-water partition coefficient generally corresponds to its octanol-water partition coefficient (K_{ow}, see glossary).

Even though bioconcentratable chemicals are often only present in water or air at trace [less that a part-per-million (ppm)] or ultra trace [less that a part per trillion (ppt)] levels, they can accumulate to toxicologically significant levels in the fatty tissues of exposed organisms. Because of this concentration process, the fatty tissues and organs of some exposed organisms can have residue levels many thousands of times higher than those found in the ambient environment (water or air). In some cases, contaminants can reach harmful concentrations in tissues of aquatic organisms even though standard analytical procedures may fail to detect residues of the chemicals in samples of the exposure water. This problem arises because analysis of water samples generally involves extraction of less than 4 liters of water at one point in time

(grab sample), whereas, for some bioconcentratable chemicals, each gram of an aquatic organism's tissue may have effectively extracted the chemical from 50 liters of water over a period of weeks or months, before reaching steady state with the exposure water. Thus, the amount of chemical residue sequestered by 10 g of fish or shellfish tissue (a typical sample size) may be 125 times greater than that found in extracts of a 4-liter water sample. Also, the composition of the chemical residues in the tissue sample may differ markedly from that found in the water sample because of possible periodic or episodic changes in water residue composition and the ability of organisms to depurate or eliminate contaminant residues.

In this primer, the basic principles of bioaccumulation are examined, the reader is familiarized with terminology related to the subject, and key factors governing bioaccumulation are described, which include chemical properties, and characteristics of the environment, the organisms, and the food chains. Also, several assessment options, including SPMDs, are described for testing for the presence of bioaccumulative substances. The focus of this primer is primarily on aquatic organisms; however, much of this information applies to terrestrial organisms as well. The chemicals emphasized in this work are the polycyclic aromatic hydrocarbons (PAHs), and in particular, the priority pollutant PAHs. The priority pollutant PAHs are sixteen compounds selected by EPA as representative of the large PAH chemical class. The selection criteria used by EPA were based on toxicity, availability of chemical standards, frequency of environmental occurrence, and chemical production data.

Section 2
FACTORS AFFECTING BIOACCUMULATION

Four major interrelated factors affect the bioaccumulation of chemicals by aquatic organisms. These include the physical-chemical properties of the contaminant, environmental conditions, and characteristics of the exposed organism and its food chain. It is important that the reader is aware that the separation of these factors is for descriptive purposes only and is not meant to imply that they are independent of each other. Also, it should be emphasized that, regardless of how low a chemical's water solubility is and how large its affinity is for sediments, there is always some exchange with other environmental compartments (e.g., water and biota), i.e., contaminant residues should be viewed as dynamic and not "fixed" even when the residence time in a compartment is great.

PHYSICAL-CHEMICAL PROPERTIES

The nature of individual atoms and the chemical bonds in organic molecules (i.e., chemical structure) confers properties of nonpolarity, lipophilicity, environmental bioavailability and mobility, and resistance to degradation. Note that in the following discussions, it is assumed that a chemical must be dissolved in water before it can be bioconcentrated by aquatic organisms.

Polarity

The presence of one or more polar functional groups (see glossary) in molecules, such as those in free acids and phenols, increases the water solubility of a compound, but reduces its K_{ow} or lipophilicity (Figure 2-1). On the other hand, the lack of polar functional groups, or the state of nonpolarity, decreases the water solubility of a chemical, and increases the lipophilicity. Also, decreasing the water solubility of a chemical enhances its escaping tendency (fugacity) from water by volatilization, unless the molecular weight is relatively high (> 350 daltons). Note that large molecules are

less volatile and thus have lower fugacities. In summary, compounds with polar functional groups generally bioaccumulate to a lesser degree than nonpolar lipophilic chemicals.

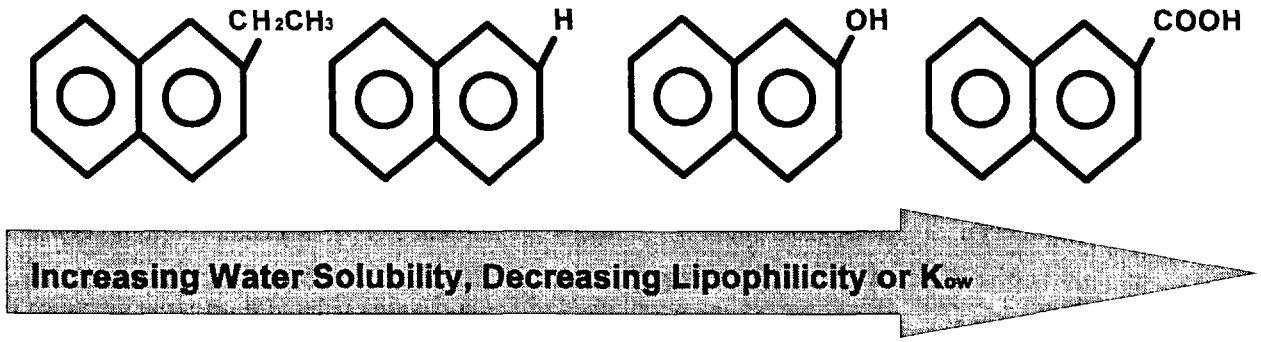

Figure 2-1. Effects of increasingly polar substituents on the water solubility and lipophilicity of organic compounds.

Molecular Size

Increasing the molecular sizes of organic compounds generally results in higher lipophilicities or K_{ow} (e.g., each nonpolar methyl group attached to a PAH molecule increases the K_{ow} by about three fold, and each nonpolar phenyl or aromatic ring increases the K_{ow} by about ten fold) and lower water solubilities (Figure 2-2). This type of molecular-size increase confers greater bioaccumulation factors (unless accumulated chemicals are metabolically altered and excreted) in organisms. However, when molecular size and K_{ow} become very large, a decline in bioaccumulation occurs, probably because of such factors as reduced water and lipid solubility, a high degree of association with natural organic matter in water, and the restricted ability of these molecules to penetrate or permeate biological membranes and access lipids in tissues. Figure 2-3 shows the sizes of several contaminant molecules relative to the postulated pore size of a fish gill membrane (Opperhuizen *et al.*, 1985) and the contaminant storage lipid, triolein. Note that the magnitude of a molecule's breadth or second largest dimension (Figure 2-3) appears to be an important factor in membrane

permeability. Also, see additional details on biomembranes in the section on "Uptake from Water," page 2-13.

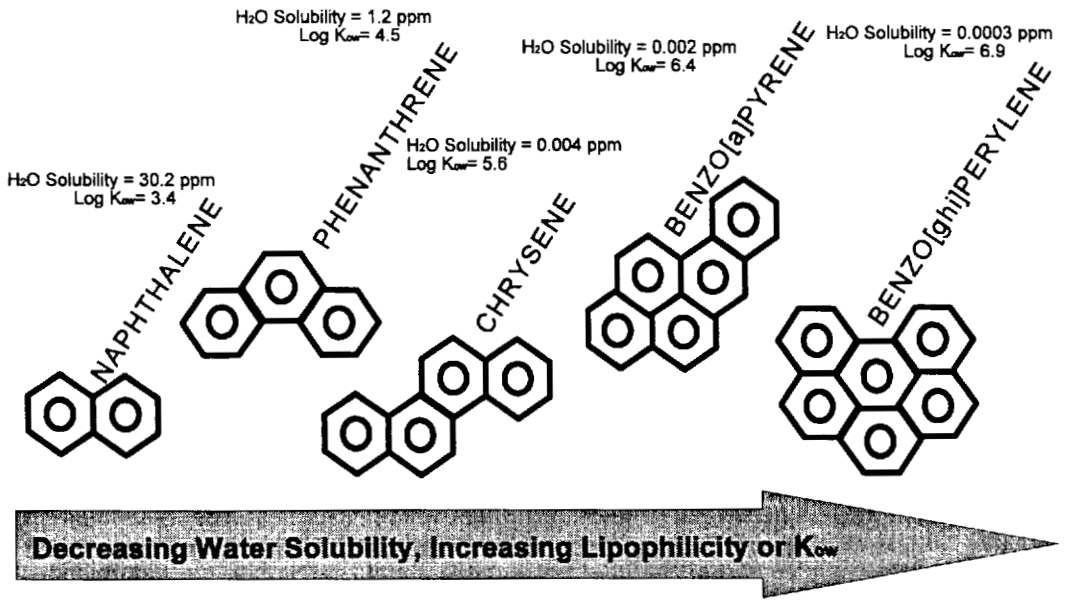

Figure 2-2. Effects of increasing molecular size on the water solubility and lipophilicity of organic compounds (PAHs).

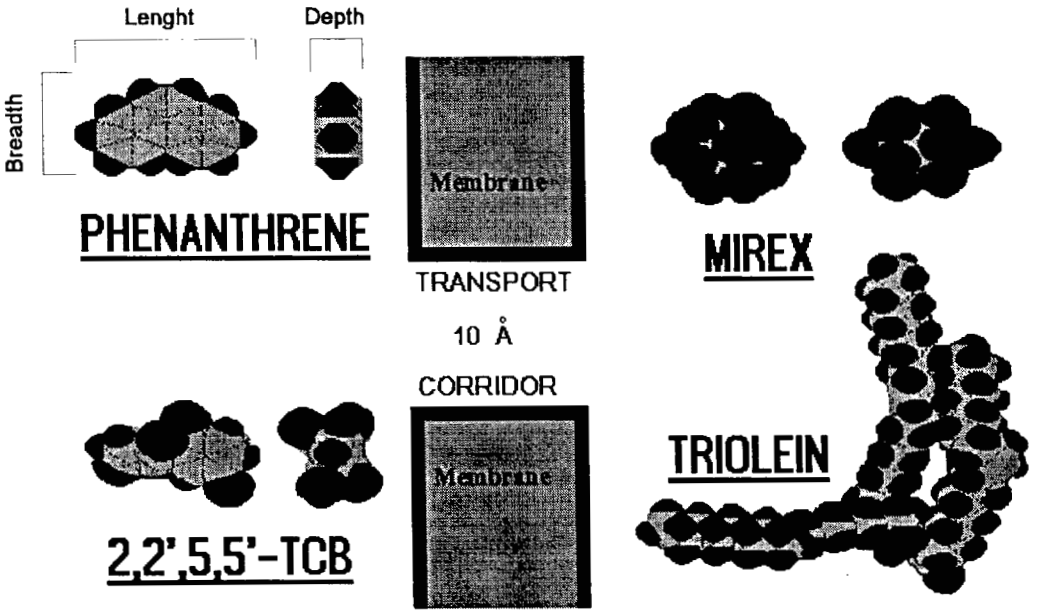

Figure 2-3. Molecular size of contaminants and a lipid relative to the postulated pore size of a fish gill. TCB is tetrachlorobiphenyl.

Table 2-1. Selected Characteristics of Organic Chemicals that Affect Bioaccumulation [modified from (Connell, 1990).

Property	Features conferring bioaccumulation
Chemical structure (strictly speaking, all other molecular properties are derived from structural features)	*High BAF*[1]: high proportion of C-C (aliphatic), C=C (aromatic), C-H and C-halogen bonds *Limited BAF:* low proportion of the bonds above with the presence of variety of polar functional groups
Molecular weight	Greater than 100, having a maximum BAF at about 350, then declining to very low BAF at about 600
Molecular dimensions	Cross sectional diameter[2] <10 Angstroms or 1 x 10^{-9} meters, molecular surface area between 208 and 460 square Angstroms, and molecular volume between 260 and 760 cubic Angstroms
Stability	Resistant to degradation reflected in soil and sediment persistence in the order of years
Log K_{ow} (lipophilicity)	Greater than 3.5, having a maximum BAF at about 6 and declining to very low BAF at about 8
Water solubility (ppm)	Less than 50, having a maximum BAF at about 0.001 with declining BAF at lower values
Degree of ionization	Very low, neutral

[1] Bioaccumulation factor

[2] The second largest dimension of a box (breadth) that will accommodate a molecule which is in a relaxed or minimized state.

Summary

Table 2-1, lists a number of molecular characteristics of contaminants that can lead to or limit bioaccumulation. The molecular structure of a chemical plays a central role in its environmental behavior. In fact it is this premise that is the basis of the science of "quantitative structure-activity relationships" (QSAR). However, the reader should keep in mind that leading authorities in QSAR research state that exact predictions of biological activity from chemical structure is always "on shaky ground" (Hansch and Leo, 1995). Nevertheless, bioaccumulation is among the more predictable forms of biological activity using QSARs.

ENVIRONMENTAL VARIABLES

The presence of lipophilic chemicals in an environment or an effluent does not necessarily mean that they will be significantly bioaccumulated. Several steps must occur in the bioaccumulation of contaminant residues, but the initial step in uptake is often contact of the dissolved residues (see the section on "Dietary Uptake," page 2-14), with an absorbing biomembrane. The amount of chemical making contact with an organism's absorbing membranes is dependent on the fraction of chemical in the exposure water that is available for uptake, i.e., the environmental bioavailability or exposure. Also, as discussed earlier, contact of a chemical with an absorbing membrane does not necessarily mean that it will readily diffuse through the membrane.

Overview of Variables

Table 2-2 summarizes several environmental factors known to affect bioavailability. Increases in these factors, with the exception of turbation of bed sediments, lead to the reduction or attenuation of the bioavailability of a chemical, even though the total amount of chemical in the system (excluding chemical breakdown processes) may remain about the same. Temperature is not included, as its effects are complex and multidirectional. Residue bioavailability may be elevated by increased temperature because of increased molecular diffusion rates but biodegradation is also elevated. Thus, for PAHs in particular, the role of temperature in bioaccumulation is beyond the scope of this primer.

Table 2-2. Characteristics of Aquatic Environments That Impact Bioavailability/Exposure of Organic Compounds, Thus Affecting Bioaccumulation.

Increasing	Effect
Amount and quality (nonpolarity) of organic carbon (bed sediment, POC and DOC)	Reduces the fraction of chemical available to an organism (note particulate-ingesters may be exceptions)
Resuspension of bed sediment	Generally results in the release of chemicals into water column from sediment pore water and by desorption from sediment particles; may reduce photodegradation
pH (4-9)	Decreases the availability of weak organic acids such as phenols and free acids; nonpolar compounds generally not affected; may cause chemical breakdown by hydrolysis
Salinity	Reduces solubility by "salting out" dissolved residues, lowering amount of available chemical
Biological activity - oligotrophic-to-eutrophic	Enhances microbial breakdown of some chemicals
Water clarity	May increase breakdown of some chemicals (e.g., PAHs) by sunlight (photodegradation)

Organic Carbon Sorption

The particulate organic carbon (POC) in suspended sediments and in bed sediments (see K_{oc} and K_p in glossary for definition of these related terms) of aquatic systems is a major environmental sink for lipophilic compounds. This is because the sediment organic carbon has many of the same chemical characteristics as lipids (remember that "like dissolves like") and represents the largest pool of organic matter in many aquatic environments. In many cases, the largest portion of the mass of chemicals with high K_{ow} (i.e., greater than 10^6) in aquatic environments is associated with POC in bed sediments and the water column (lesser extent), thus reducing the availability of the residues for uptake by organisms. This reduction occurs even though most of the mass

of natural sediments is inorganic minerals such as silicates, which generally do not play a major role in lipophilic compound sorption. However, on a gram-(lipid)-to-gram (organic carbon of sediment) basis, the lipids of native organisms at a sample site can have higher concentrations of persistent bioconcentratable compounds (e.g., chlorinated hydrocarbons) than those found in sediment organic carbon at the same site (see Table 2-3, and references cited therein). Since the amount of sediment-related organic carbon in a natural aquatic ecosystem is generally much greater than the total mass of lipids in resident organisms, the lipids of aquatic species typically do not represent a significant environmental repository for bioaccumulative contaminants. Possible exceptions include aquaculture systems with very high biomass and some ecosystems with established populations of zebra mussel (*Dreissena polymorpha*).

Table 2-3. Relative Distribution of PAHs and Chlorinated Hydrocarbons in Bivalve (*Macoma nasuta*) Lipid (L) and Sediment Organic Carbon (OC). L/OC Values Are Determined By: (Chemical Concentration in Tissues/ Fractional Lipid Content)/(Chemical Concentration in Sediment/Fractional Organic Carbon).[1]

PAHs	L/OC Ratios	Chlorinated Hydrocarbons	L/OC Ratios
Pyrene	0.5 (n=6)	PCBs[2]	1.5 (n=12) 2.5 (n=6)
Chrysene	0.4 (n=6)	HCB[3]	2.0 (n=6)
Benz[a]anthracene	0.4 (n=6)	DDE	1.3 (n=6)
Benzo[a]pyrene	0.3 (n=6)		
	$\bar{x}$ = 0.4		$\bar{x}$ = 1.8

[1] Data from (Ferraro *et al.*, 1990; (Boese *et al.*, 1996; Rubinstein *et al.*, 1990; Lee, 1992).
[2] Polychlorinated biphenyls.
[3] Hexachlorobenzene.

In cases where the lipophilic compounds are readily metabolized or actively depurated by aquatic organisms (e.g., PAHs), sediments will often contain higher concentrations of these chemicals than organism tissues. Table 2-3 illustrates organism/sediment ratios derived by dividing concentrations of selected contaminants measured in bivalve (shellfish) lipids by the respective concentrations in the organic carbon of sediments (Boese and Lee, 1992). The greater than four-fold difference (Table 2-3) in the distribution of chlorinated hydrocarbons versus PAHs in organism lipids and sediment organic carbon lends credence to the "active or metabolic PAH elimination" hypothesis for organisms.

Chemical bioavailability and bioaccumulation are often reduced but not eliminated in environments having sediments with high organic carbon contents. For example, after episodic releases of chemicals occur, concentrations of the chemicals in overlying water eventually decline due to sediment sorption, dilution, etc. Subsequently, desorption of these sorbed chemicals from the contaminated sediments may represent a significant source of bioconcentratable chemical.

Dissolved organic carbon (DOC, see glossary) in a system also affects the bioavailability of chemicals. Although there is only an operational difference between DOC and POC [i.e., DOC is organic matter with a diameter less than 0.1 micrometers (1×10^{-7} meters), whereas POC is particulate organic matter (usually attached to inorganic mineral particles) with a diameter greater than 0.1 micrometers], the effects of these two classes of organic carbon on aquatic bioavailability can be quite different.

In some cases the DOC may represent truly dissolved low-molecular-weight biogenic molecules, such as fatty acids, alcohols, ketones, etc., which act as trace cosolvents that may slightly increase the solubility of hydrophobic chemicals in water without impeding membrane transfer or biouptake. If the DOC in an aqueous environment consists largely of humic acids and other natural macromolecules (generally greater

than 2000 molecular weight), then bioavailability is initially retarded as chemicals sorbed on this type of DOC may not make intimate contact with biomembranes. However, the rate a chemical is released from these very small natural sorbents is generally rapid when compared to release from larger-sized POC (Mackay, 1994).

When bed sediments containing chemicals are resuspended by turbulent mixing events (e.g., dredging and flooding), chemicals are released into the overlying water by desorption from the resuspended sediment particles. This phenomenon occurs because chemicals in sediments are often at steady state with sediment pore water but not with overlying water, i.e., pore water chemical concentrations are typically higher than those in overlying bulk water. Keep in mind that there is always a natural tendency for chemicals to be at steady state or in balance with the surrounding environment. Also note that the light-induced breakdown of some desorbed chemicals may be reduced or attenuated by sediment resuspension. This occurs because of absorption of sunlight by the greater concentrations of natural organic matter, as described by Lamberts Law.

Acidity

The effects of pH on the ionization of organic chemicals are generally well known. Since ions of organic compounds are typically not transported across biomembranes (see the section on "Uptake from Water," page 2-13), changes in pH that result in the ionization of organic compounds reduce bioavailability and thereby reduce bioaccumulation. On the other hand, changes in pH that reduce ionization, enhance lipophilicity or K_{ow} (see Figure 2-4) and bioavailability.

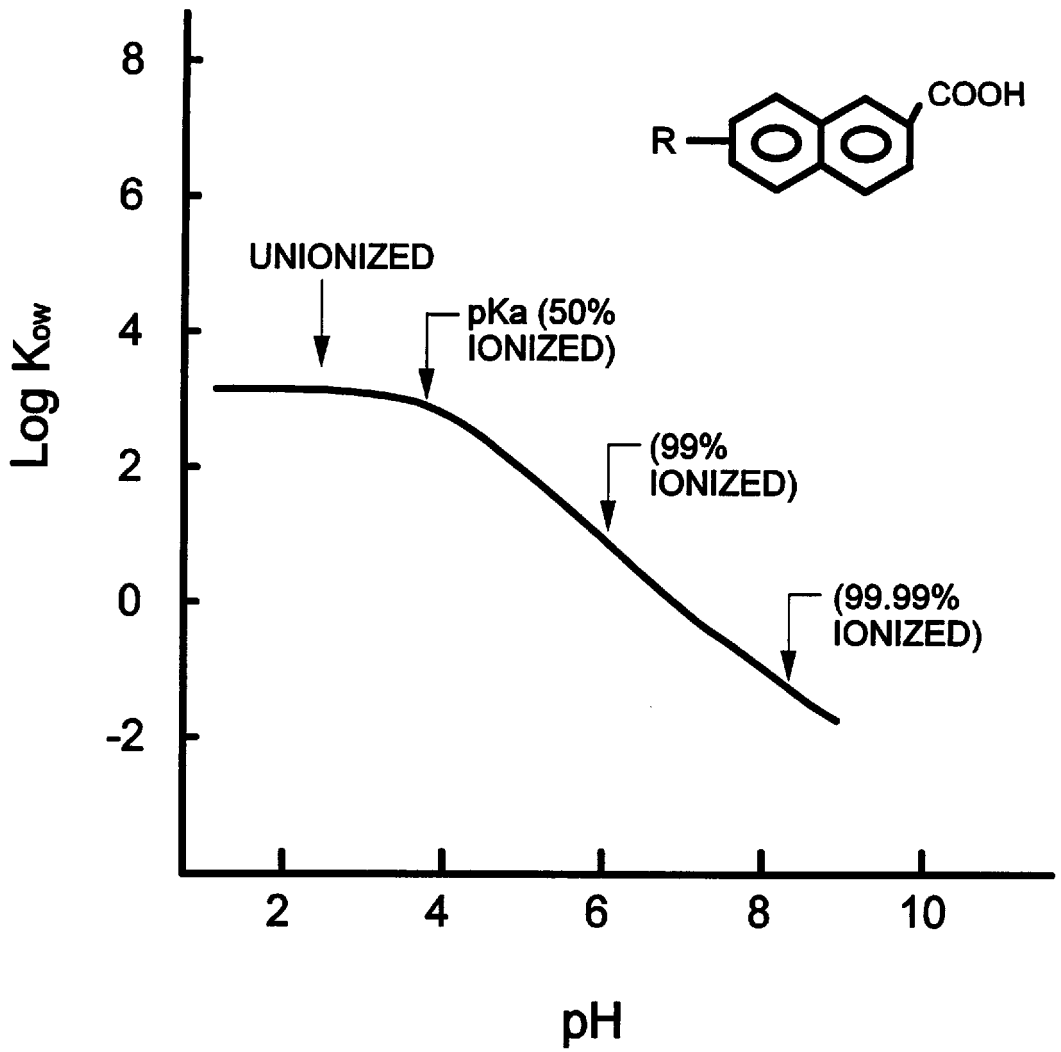

Figure 2-4. The effects of pH on the K_{ow} or the lipophilicity of a weak organic acid, where R is an alkyl group (modified from Connell, 1990). The pH at which a compound is half ionized and half unionized is denoted pKa.

Salinity

Another environmental factor that varies considerably and has a role in bioavailability is salinity. However, high salinity [i.e., 35 grams/liter (sea water)] only reduces the solubility of neutral organic compounds such as PAHs by 30 to 60% (Brown, 1978). Since marine and freshwater (low salinity) species appear to have about the same bioconcentration factors for the same chemicals (Connell, 1990), the amount of

accumulated residues from similar environmental inputs of chemicals should be about the same or only 30 to 60% less (worst case) for marine organisms.

Environmental Degradation Processes

Degradation of a bioconcentratable organic compound occurs when its structure is changed by the loss and/or gain and substitution of an atom or a group of atoms, as well as the accompanied changes in the affected chemical bonds. This applies to both biologically (e.g., microbial degradation) and chemically [e.g., light- and pH-mediated breakdown (photolysis and hydrolysis, respectively) of chemicals] driven environmental processes. There are several levels of degradation; one of these is partial where much of the parent molecule is preserved (e.g., DDT to DDE) and another is the complete breakdown of the molecule into inorganic species such as water and carbon dioxide (mineralization). Aerobic conditions (oxygenated environments) promote more complete degradation. If the degradation of a chemical is partial, then concern should shift to whether the toxicity or other undesirable characteristics, such as the propensity to bioaccumulate, have been decreased or increased in the resulting products. Note that certain light-produced breakdown products of PAHs in organism tissues and water show enhanced toxicities. Thus, the same factors that promote photolytic breakdown of PAHs have the potential for increasing PAH residue toxicity.

When compared to microbial degradation, metabolism by higher tropic level organisms such as fish and bivalves plays a much less significant role in the removal of bioconcentratable chemicals from aquatic systems. Even though compounds such as PAHs are degraded by multiple environmental processes, the continuous input of these chemicals from effluents or nonpoint sources can lead to some bioaccumulation. Also, note that residues sorbed on sediment are degraded much more slowly than the same residues dissolved in water, and thus sediment sorption increases the environmental persistence of degradable compounds.

Figure 2- 5 shows the chemical structures of selected organic compounds with low to high environmental persistence, which is often related to lipophilicity or bioaccumulation potential. However, some very persistent contaminants with high K_{ow} (e.g., mirex) have relatively low BCFs, which have been attributed to reduced water and lipid solubility, sediment sorption, and possibly low biomembrane permeability.

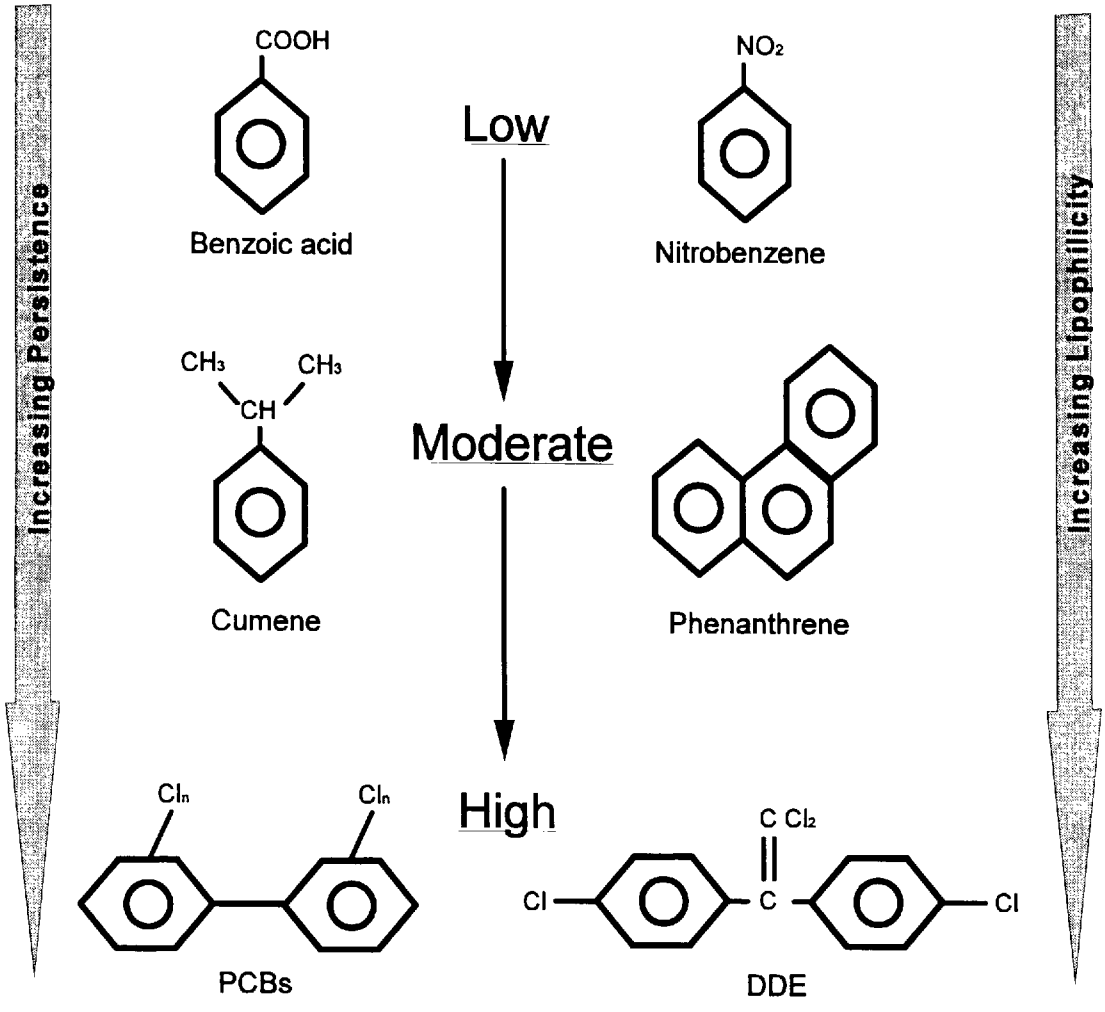

Figure 2-5. Chemical structures of selected organic compounds having increasing environmental persistence and lipophilicity. Note that high environmental persistence does not always lead to large BAFs.

ORGANISM-RELATED FACTORS

Lipophilic contaminants are accumulated by aquatic organisms from water via respiration and from ingested food or sediments as illustrated in Figure 2-6. Both of these routes of uptake are significant for many aquatic organisms, with the exception of phytoplankton or photosynthetic organisms that do not ingest food. However, bioconcentration (uptake from water) is generally viewed (Connell, 1990; Barron, 1990) as the dominant route of uptake for most chemicals (including most PAHs) by aquatic organisms. Diet is most likely to be a major route of uptake when chemicals are persistent and have high K_{ow} (i.e., greater than 10^5). This is especially true for organisms that are air-breathing, long-lived, and are top predators (aquatic and terrestrial). However, in most cases the organisms at the base of food chains, e.g., photosynthetic phytoplankton, only accumulate chemicals directly from water. Thus, even the dietary route of chemical uptake for most organisms is ultimately based on direct uptake from water.

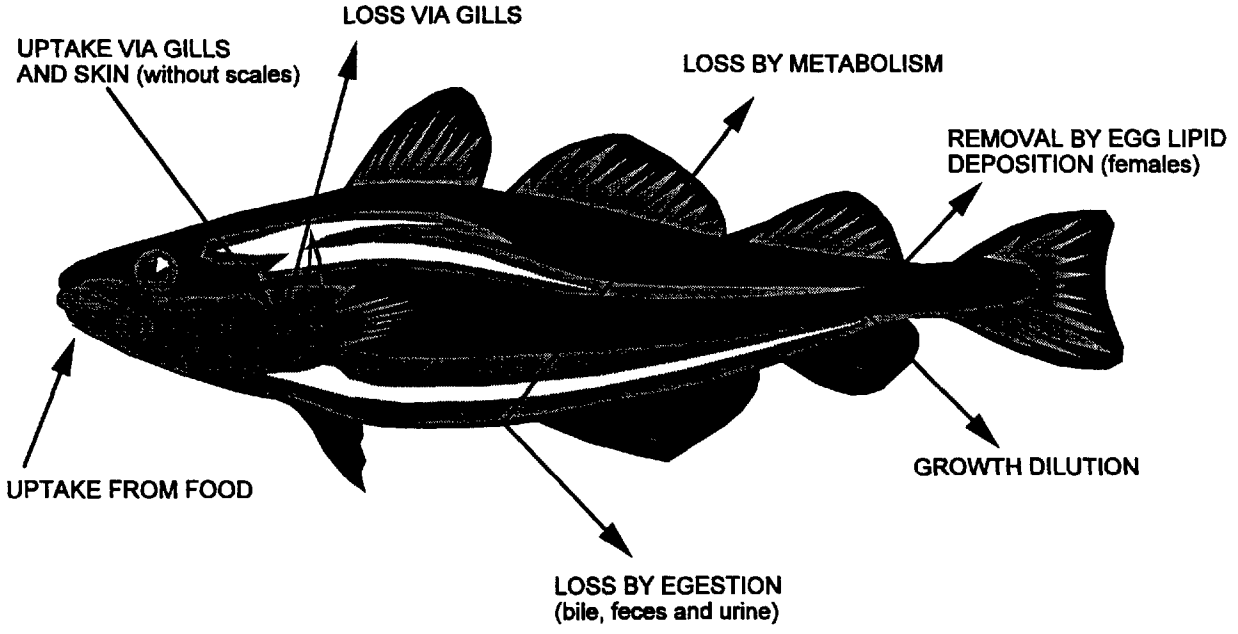

Figure 2-6. Multiple routes of chemical uptake, elimination and growth dilution exhibited by various aquatic species.

Uptake from Water

Relatively large volumes of water (often liters per day) are ventilated across the gills of fish and bivalves for respiration and feeding (bivalves). In order for an organic compound to be extracted from ventilated water, it must passively diffuse through several barriers between the ambient water and lipid storage sites in the organism. The barriers include an exterior stagnant water layer, a mucus layer, and the absorbing bilayer membrane or gills. After passing through these barriers, residues are then transported via blood to fatty tissues, where they are concentrated. One note of interest is that the lipid content of the organism has little or no effect on uptake rates but it does affect the capacity of an organism to bioconcentrate a chemical.

The gill bilayer membrane is composed of a lipid matrix with embedded proteins that have receptors and enzymes. Lipophilic organic compounds diffuse through the fatty regions of gill cell membranes (Barron, 1990) rather than moving through intercellular channels (spaces between cells) in the bilayer membrane where specific inorganic ions are actively transported to maintain homeostasis. The average distance from the external water to an organism's blood (across the aforementioned barriers) is only about 6 micrometers (Hayton and Barron, 1990). Because the matrix of the cell membrane has extremely limited free space, a molecular size limit (breadth or cross sectional diameter) of about 10 Angstroms has been suggested for bioconcentratable compounds (see Figure 2-3). Since most environmental contaminants are small enough to diffuse through cell membranes, these barriers can impede, but cannot exclude the uptake of most lipophilic contaminants. For example, McKim *et al.* (1985) have shown that the assimilation efficiency of a variety of lipophilic compounds across fish gills ranges from about 20 to 90% of the contaminant residues present in ventilated water.

Dietary Uptake

Many of the same considerations discussed above apply to dietary uptake or the assimilation of chemicals across the gut. However, several differences do exist between the dietary and respiratory uptake of chemicals. If ingested materials are

largely nondigestable such as most sediments [certain polymeric fractions (e.g., humin) of sediment organic carbon are highly resistant to digestive chemical attack], then the likelihood of gastrointestinal uptake is diminished by the lack of direct contact of the contaminant with the absorbing gut membrane. In any case, desorption of chemicals from ingested organic matter must occur for chemical uptake.

For example, the gut assimilation efficiency of hexachlorobenzene (HCB) from ingested sediments was only 15% for a sea cucumber (Ekelund, 1989), whereas about 50 to 85% of the PCBs in fish food were assimilated across the gut of fish (Thomann *et al.*, 1992). Since HCB and PCBs have similar physiochemical properties (e.g., the log K_{ow} for HCB is about 6.4 and the mean log K_{ow} of the PCB mixture, Aroclor 1254, is about 6.3) and the biological membrane transfer efficiencies from the same matrix are nearly identical (McKim *et al.*, 1985), the observed difference in HCB and PCB gut assimilation efficiencies from sediment and food is likely due to the low escaping tendency (low fugacity) of HCB residues from sediments, even during digestion. As suggested above, the fugacity of high K_{oc} contaminants in sediments generally remains low during digestion, because there is little change in the pollutant-containing organic carbon matrix and, unlike some organic solvents, the extraction efficiency of digestive fluids for sediment-sorbed residues appears to be low.

It has been postulated that fat droplets or micelles (generated during digestion) that contain contaminants can be actively transferred across the gut membrane without having to diffuse through cell wall matrix. However, the findings of Bruggeman *et al.* (1984) and Zitko and Hutzinger (1976) strongly suggest that fat-mediated transfer across the gut does not occur. Thus, about the same molecular size limitation postulated for respiratory membranes appears to apply to gastrointestinal membranes.

From the data available in the literature (Connell, 1990; Pruell *et al.*, 1986; Ekelund, 1989; Thomann *et al.*, 1992) there appears to be large differences in gut assimilation

efficiencies (i.e., 4 - 90%) among different species for the same chemicals. This is particularly true for PAHs, as the gut assimilation efficiencies of the same PAHs by polychaete worms, fish, and crustaceans range from very low (less than 10%) to large (greater than 70%), with great variability among fish species. Again, part of the reason for this variability in gastrointestinal assimilation efficiencies across species may stem from species-to-species differences in the degree of breakdown of ingested organic matter.

Depuration of Accumulated Residues

Bioaccumulation of contaminant residues occurs only if the rate of a chemical's uptake exceeds the rate of its elimination. Both the toxicity and bioaccumulation potential of a contaminant are greatly affected by the rate of elimination from an organism. If an unaltered chemical can be eliminated rapidly, residues will not accumulate and tissue damage is less likely. In aquatic organisms, depuration of many lipophilic chemicals occurs passively across the gills, and in some cases the skin (fishes without scales, such as catfish), by the reverse of the diffusion-partitioning process involved in uptake (Figure 2-6). The physical characteristics of the gill make it the principal organ for elimination of this type. Gill elimination appears to be most important for nonpolar compounds that are not rapidly biotransformed. The rates of elimination for these compounds are generally inversely related to K_{ow} (Spacie and Hamelink, 1985) unless metabolism is the major route of elimination.

In the case of lipophilic contaminants that are readily metabolized, such as PAHs, the major route of elimination in fish and other vertebrates is by the bile. Metabolites of PAHs and other chemicals are usually formed in the liver and transported to the gallbladder, where they are discharged with the bile into the gut and eliminated in the feces. These polar metabolites have much greater elimination rates than the parent compound. For PAHs and several other types of contaminants, residue analysis of the bile may provide more information on chemical exposure than residue analysis of whole body tissues. In general, urinary excretion appears to be a much less significant route of lipophilic contaminant elimination.

Two other elimination processes are also important. Fish, birds, and invertebrates eliminate lipophilic chemicals through egg deposition (Derr and Zabik, 1974; Guiney, *et al.*, 1979) and mammals eliminate chemicals by lactation and via the fetus (Thomann *et al.*, 1992). Obviously, gender must be considered when BAFs are estimated.

Metabolism and elimination of PAHs by invertebrates are quite variable. The hepatopancreas (a digestive gland that combines the function of the liver and pancreas in invertebrates) plays a major role in the metabolism of PAHs by many crustaceans but not in others. Typically, the distribution of PAH-metabolizing enzymes in invertebrate tissues differs markedly from that of vertebrates.

In many organisms (especially mammals, birds, and aquatic vertebrates) the enzyme system variously known as the cytochrome P-450-dependent mixed-function oxidase [the mixed-function monooxygenase (MFO)] system, and the aryl hydrocarbon hydroxylase system is responsible for the biotransformation of a variety of lipophilic compounds, especially the PAHs. In fish, birds, and mammals most MFO activity is localized in the liver, and the route of elimination from the liver is by the bile as described earlier.

Although the MFO system effectively detoxifies some contaminants, others such as certain PAHs and alkanes can be transformed to intermediates that are more toxic than the parent compound. For example, the carcinogenicity of benzo[a]pyrene, a PAH, is thought to be due to an MFO-generated metabolite of the parent compound. Invertebrates generally appear to have lower levels of MFO activity; this is particularly true for bivalve molluscs, which accounts in part for their popularity as biomonitoring or sentinel organisms. In summary, biotransformation or metabolism often plays a dominant role in limiting bioaccumulation, especially for PAHs.

Toxicity and Bioaccumulation

Even though the focus of this document is bioaccumulation, some limited observations are in order on how bioaccumulation or the lack thereof relates to the toxicity of PAHs. In short-term or acute exposures, PAHs exhibit only limited toxicity to aquatic organisms, even when concentrations approach water solubility (Neff, 1985). This acute mechanism of PAH toxicity appears to be due to disruption of plasma membrane function (Neff, 1985) and is directly dependent on the BAF.

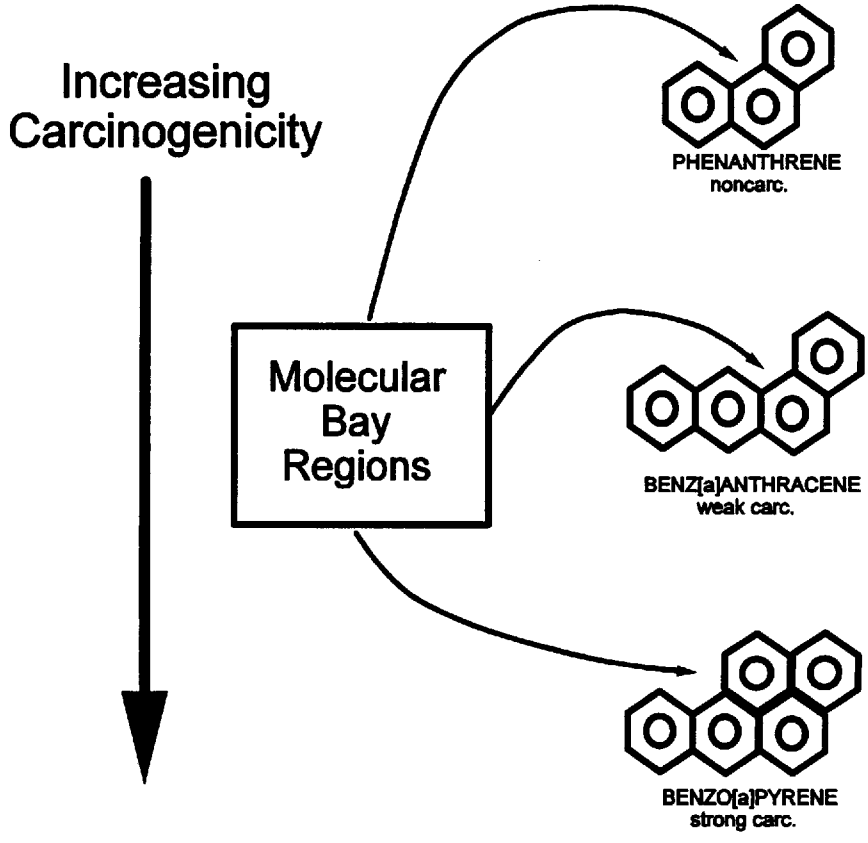

Figure 2-7. Selected PAHs, having bay regions in their molecular structure, and their relative carcinogenicity.

Certain types of PAHs exhibit more structure-specific forms of toxicity which include carcinogenicity and photo-induced toxicity. Both of these types of effects have been the subject of considerable QSAR research. A key ingredient of PAH carcinogenicity

appears to be the presence of a bay region in the PAH molecule. Figure 2-7 shows the structures of several PAHs, illustrates what a bay region is, and provides information on the relative carcinogenicity of the compounds shown. Having a bay region in the molecule's structure appears to be a requirement for carcinogenicity but it does not necessarily mean that all PAHs with bay regions are carcinogenic. Bay region theory is beyond the scope of this primer and has been thoroughly discussed by Lehr *et al.* (1985). However, keep in mind that PAH carcinogenic activity requires MFO or metabolic activation to more polar transformation products, which reduces bioaccumulation of the parent molecule.

Another structure-specific concern about PAH environmental residues is photo-induced toxicity. Studies have shown that, in the presence of UV light, anthracene and some other PAHs can be as much as 1000-fold more toxic to aquatic organisms (Veith *et al.*, 1996). This photo-induced toxicity applies to residues accumulated within certain organism tissues (McKenyan *et al.*, 1994) and residues dissolved in shallow waters. The magnitude of the effects on organisms with accumulated PAHs is directly related to the BAFs of the photo-active PAHs. Again, description of this molecular structure-toxicity relationship or QSAR is beyond the scope of this document, but it has been examined in the literature (Veith *et al.*, 1996; McKenyan *et al.*, 1994) and is known to relate to the so called HOMO-LUMO gap (energy difference between lowest and highest occupied molecular orbitals) of the PAH molecule. Of EPA's priority pollutant PAHs, the following have been found to be phototoxic: anthracene, pyrene, benz[a]anthracene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene and benzo[g,h,i]perylene. The addition of hydrocarbon substituents or groups, e.g., CH_3 or C_2H_5, appears to have little effect on the photo-induced toxicity of a chemical, but the potentially higher BAFs of PAHs with nonpolar substituents may increase the risk of effects due to higher tissue concentration of toxic photo-degradation products.

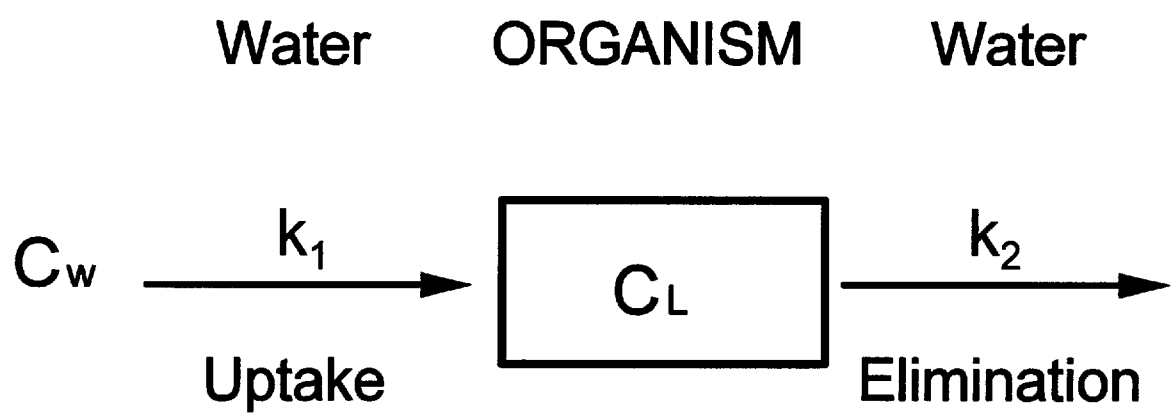

Figure 2-8. Single-compartment model for the uptake and elimination of lipophilic chemicals by an organism, where C_W is chemical concentration in water, C_L is chemical concentration in the organism's lipid, and k_1 and k_2 are rate constants.

Kinetic Models of Bioaccumulation

Although bioaccumulation is a dynamic and complex process, the bioconcentration portion (uptake from water) of the bioaccumulation process can generally be modeled by a simple, one-compartment kinetics model (Figure 2-8). A compartment model is a mathematical description of the quantity of a chemical within a uniform component or matrix, which is determined by competing rates of chemical uptake and elimination. For example, envision a single fatty cell, such as a diatom, in which bioconcentration is governed by a chemical's passive diffusion through the cell membrane and its partitioning between the cell's lipid and the surrounding water. In this case the rate of change of residue concentration in the cell lipid over time is given by

$$dC_L/dt = \text{uptake} - \text{loss} = k_1C_w - k_2C_L \qquad \text{(Equation 2-1)}$$

where k_1 is the uptake rate constant, C_w is the concentration of the chemical in water (dissolved), k_2 is the first-order elimination or depuration rate constant, and C_L is the concentration of the chemical in lipid. Generally speaking, the concentration of a chemical in an organism is expressed in terms of whole body concentrations, C_t, rather

than normalized to lipid content, C_L, as in Equation 2-1. After a sufficient exposure duration (days to months), concentrations in tissues approach steady state with the surrounding water. Thus

$$dC_t/dt = k_1C_w - k_2C_{tss} = 0 \qquad \text{(Equation 2-2)}$$

where C_{tss} is whole body residue concentrations at steady state and then

$$BCF = C_{tss}/C_w = k_1/k_2 \qquad \text{(Equation 2-3)}$$

Upon integration of Equation 2-1, one can determine C_t at any point in time (t) by

$$C_t = k_1/k_2\ C_w[1-\exp(-k_2t)] \qquad \text{(Equation 2-4)}$$

Note that BCF may be substituted for k_1/k_2 in Equation 2- 4 and that this equation describes a first-order rise to steady-state tissue concentrations, i.e., $C_t \rightarrow C_{tss}$. Examination of Equation 2-4 reveals that its amplitude is set or limited by the BCF (k_1/k_2) of a chemical, and the time required to reach steady state is dependent on the magnitude of k_2. The assumptions used in deriving these one-compartment models are that uptake is solely from water, that the water concentration remains constant, and that the compartment is homogeneous with respect to chemical concentrations.

Model Terms and Concepts

To have a conceptual understanding of how these models relate to the bioconcentration process, it is useful to examine the nature of k_1 and k_2. The uptake rate constant k_1 has units of milliliters (mL)/(day x gram of organism tissue). Thus, k_1 can be viewed as the effective total volume (mL) of water extracted of chemical by a gram (g) of organism tissue in one day (d). Another way to view k_1 (Spacie and Hamelink, 1992) is by

$$k_1 = ER_v/M \qquad \text{(Equation 2-5)}$$

where E is the extraction efficiency of a chemical by the gill, R_v is the volume of water ventilated or passed over the gill in one day, and M is the mass of the fish tissue.

From the literature (Mackay *et al.*, 1992), the magnitude of priority pollutant PAH k_1 or uptake rate constants for several invertebrates (very few vertebrate k_1 values have been reported) range from about 800 to 5,000 mL/day x gram. Again note that the uptake rate constant is independent of an organism's lipid content.

The units of the first-order depuration rate constant k_2 are the reciprocal of time or t^{-1} (which is difficult to fathom). However, another way of viewing k_2 is the constant fraction or portion of a chemical's remaining concentration in an organism's tissue that is eliminated on a daily basis. Obviously, k_2 reflects the net fractional amount of chemical eliminated by the potentially multiple depuration processes available to organisms, as described earlier. If plots of the chemical concentration remaining in tissues versus time do not follow first-order kinetics, which require that the fraction or percent of chemical eliminated per unit time remains constant regardless of the tissue concentration, then more complex models (e.g., two compartment models) must be used. An example of this situation can be envisioned by assuming that a portion of the residues accumulated by an organism resides in a separate compartment, where the chemical has a much higher affinity for the lipids present, and thus this portion of residues is mobilized and eliminated at a much slower rate.

Application of Models to Biological Data

To verify that Equation 2-4, is applicable to field data on the uptake of chemicals by transplanted or unexposed organisms, a plot of time (x-axis) versus the natural log of the concentration (y-axis) of the chemical in exposed tissues should be linear. This is the standard test for first-order kinetics. Assuming that the model fits the overall uptake data and that depuration is also first-order, then the half-lives of tissue residues can be

estimated by $0.693/k_2$, and four half-lives will reduce the tissue's concentrations by about 95%. However, it is always prudent to confirm residue half-lives by directly measuring the depuration of accumulated residues.

Half-Lives of Bioaccumulated PAHs

In the case of PAHs, Lee *et al.* (1978) exposed oysters (*Crassostrea virginica*) to crude oil enriched with several PAHs in seawater. Some of these exposed oysters were subsuently placed in clean seawater and the half-lives of selected PAHs were determined in organism tissues. The oyster tissue half-lives of naphthalene, anthracene, fluoranthene, benz[a]anthracene and benzo[a]pyrene (BaP) were 2, 3, 5, 9 and 18 days, respectively. The half-lives increased with the lipophilicities or K_{ow} of these compounds, which follows partitioning theory for non-metabolized chemicals. The k_2 values ranged from 0.34 to 0.043 day^{-1}. From these data and other marine bivalve data, Neff (1985) concluded that molluscs are able to depurate the majority of accumulated PAH residues from tissues within several weeks. By comparison, a PCB [2,2',5,5'-tetrachlorobiphenyl (TCB)] had a half-life in the guppy fish of 43 days (Connell, 1990), whereas BaP had a half-life of 18 days in the oyster tissues (Lee and Gardner *et al.*, 1978). The lipophilicities of these two compounds are about the same (i.e., BaP's log K_{ow} = 6.35 and TCB's log K_{ow} = 6.02), and fish generally have more MFO activity or greater ability to metabolize chemicals than oysters. These and other data strongly suggest that aquatic organisms have a much greater capacity to degrade and/or eliminate PAHs than more persistent compounds such as PCBs.

Thus, the elevated k_2 or depuration rate constants of PAHs measured in many organisms appear to be due to active (e.g., export of metabolites or parent compound with feces) as opposed to passive (e.g., outward gill diffusion) partitioning elimination mechanisms. Further support for active depuration of PAHs is found in the half-lives of PAHs in the bivalve mollusc (*Mytilus edulis*), as reported by Pruell *et al.* (1986). In this case, the half-life of benz[a]anthracene ($K_{ow} = 8 \times 10^5$) was 17.8 days, and the half-life

of benzo[g,h,i]perylene (K_{ow} = 7.9 x 10^6) was 15.4 days. Since the lipophilicities (i.e., K_{ow}) of these two compounds differ by about ten-fold, it seems likely that active depuration must have occurred in the case of benzo[g,h,i]perylene.

As mentioned earlier, crustaceans depurate PAHs at rates higher than bivalve molluscs. Southworth *et al.* (1978) measured half-lifes of PAHs (naphthalene, phenanthrene, anthracene, 9-methylanthracene, pyrene, benz[a]anthracene and perylene) in the freshwater crustacean *(Daphnia pulex)*. They ranged from 0.4 hours to 5 hours. Comparison of these data to the bivalve data examined illustrates the large differences in the abilities of aquatic organisms to depurate PAHs. If the half-life of a chemical is less than one week, then chronic toxicity is generally not a concern. Note that exceptions include cases where there are continuous chemical inputs or the chemical is a carcinogen.

In summary, the ability to eliminate accumulated PAHs varies greatly among species but generally follows:

mammals (high) > fishes (relatively high) > crustaceans (moderate) > bivalve molluscs (low)

Because aquatic organisms generally have rapid depuration rates of PAHs, flow-through exposures to measure BCF and BAF can often be limited to four weeks or less, with some confidence that steady-state tissue concentrations will be achieved.

Estimation of Potential BCF Using QSAR Models

Since the same PAHs are differentially eliminated by many organisms, the use of K_{ow} or hydrophobicity as a means of predicting a PAH's BCF (standard QSAR) must be approached with extreme caution, and low accuracy (may be off by greater than an order of magnitude) can be expected, especially when applying this type of modeling

approach across species. Also, if BCF data are used that are not measured at steady state, the model fits will be further compromised. Southworth *et al.* (1978) developed the regression model (a statistical model best fitting the data)

$$\log BCF = 0.75 \log K_{ow} - 0.44 \qquad \text{(Equation 2-6)}$$

for uptake of PAHs by *Daphnia pulex,* as described earlier. The highest observed BCF (whole tissues) in this study was about 10^4 for benz[a]anthracene. After normalizing the BCF to an approximate 2% lipid content of the daphnia tissues, i.e., 10^4 (BCF)/0.02 (lipid content) = 5×10^5, the resulting BCF is half the K_{ow}. This value suggests that equilibrium was not achieved or that active depuration or metabolism occurred, because BCF/K_{ow} is significantly less than one. When BCFs are measured correctly, the BCF/K_{ow} ratio approach, i.e., lipid normalized BCF/K_{ow}, provides insight into a chemical's or a class of chemicals' relative potential for bioaccumulation and food chain transfer.

FOOD CHAIN-RELATED FACTORS

Theory

Until recently, measured contaminant biomagnification factors (i.e., increasing BAFs of certain chemicals in organisms occupying higher tropic position in a food chain) in the literature did not appear to be compatible with existing bioaccumulation theory. Connolly and Pedersen (1988) hypothesized that food chain transfer and thus biomagnification result from a fugacity (escaping tendency) gradient established in the gut of the animal as contaminated food is digested. This hypothesis is now widely accepted as the basis for the biomagnification phenomenon. It is useful to examine what Connolly and Pederson(1988) meant by the "fugacity gradient" and describe the sequence of events that must occur for biomagnification. Contaminants in the lipids of prey, such as crustaceans, are generally at steady-state with the surrounding waters. As these prey tissues are digested in the gut of a predator, such as a small fish, the

lipids are broken down into more polar constituents, thus reducing the capacity of the digestate to solubilize or retain the nonpolar contaminant residues. This high escaping tendency or fugacity of nonpolar lipophilic contaminants from digested materials results in a net transfer of these chemicals into the predators lipid-containing tissues. Then, assuming that the predator continues to consume numerous prey, the rate of active uptake from the diet can exceed the rate of passive elimination into the water, resulting in contaminant concentrations higher than those that would be found in its fatty tissues at equilibrium. If this animal is, in turn, consumed by a higher trophic level species, a further magnification in residue concentrations can occur. This is the theoretical basis for biomagnification, and the phenomenon has occurred if BCF/K_{ow} is significantly greater than one. The likelihood of the occurrence of this phenomenon in environmental food chains is greatly reduced if a compound is readily metabolized, actively depurated, or its K_{ow} is less than 10^5.

Biomagnification

Assuming a chemical of interest is not metabolized or actively depurated, then food chain transfer must be considered. In an EPA guidance document on "Assessment and Control of Bioconcentratable Contaminants in Surface Waters" (Burkhard *et al.*, 1991), BAFs (including the effects of biomagnification) are determined by taking the BCF and multiplying it by a food chain multiplier (FM), i.e., BAF = BCF x FM. The recommended food chain multiplier ranged from 1 to 104, depending on the compound's K_{ow} and the trophic level. The trophic levels covered in the guidance document were 2 through 4, where level 2 is an omnivorous (plant and animal consumer) invertebrate or a very small fish; level 3 is a lower carnivore, typically a small fish; and level 4 is a top carnivore fish of medium to large size.

This food chain transfer scenario does not include fish-eating birds or aquatic mammals, which generally have an even higher FM value than that given for the level 4 example. From the EPA document (Burkhard *et al.*, 1991), the food chain multipliers for

a nonmetabolized compound with a K_{ow} of 10^6 would be 6.8, 21.4 and 66.5 for trophic levels 2, 3, and 4, respectively. Thus, if the BCF estimated for a chemical is 10^6, after normalizing the whole body tissue value to the lipid content of an organism (as described earlier), then a large predator fish (level 4) may have an extremely high BAF of 6.65×10^7. Figure 2-9, illustrates the increased concentration of a contaminant with trophic level.

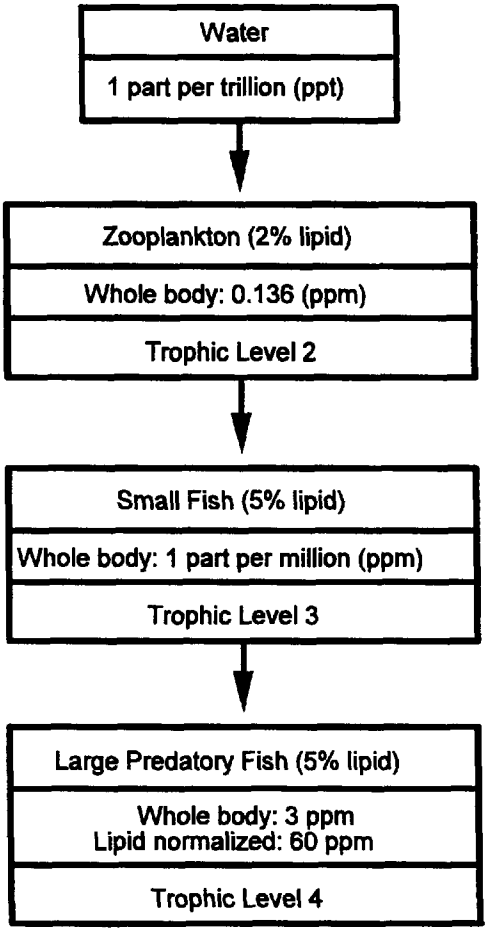

Figure 2-9. Food chain biomagnification of a typical PCB ($K_{ow} = 10^6$). The BAF, based on lipid in the tropic level 4 predator, is 6.0×10^7. Note that trophic level 1 (phytoplankton) is not included in this example.

An Example

A vivid example of how food chain effects on the BAF of a contaminant can lead to environmental problems has been reported for Lake Laberge in Canada's Yukon territory (Kidd *et al.*, 1995). Very high concentrations of the banned pesticide toxaphene have been found in the tissues of large fishes from the lake, whereas fishes from neighboring water had only barely detectable amounts of toxaphene. The amounts of toxaphene in the waters of Lake Laberge and other nearby lakes were extremely low with no detectable differences. Investigators (Kidd *et al.*, 1995) now believe the sole reason for this BAF problem is the very high consumption rate of especially fatty prey fish (different than the other lakes) by the top predator fishes, i.e., food chain biomagnification.

In general, the possibility of biomagnification of PAHs up the food chain is very low when compared to chlorinated hydrocarbons. However, because of large interspecies differences in the ability of organisms to metabolize PAHs, the described fugacity gradient process could contribute to the bioaccumulation of PAHs in some organisms.

Section 3
APPROACHES FOR ASSESSMENT

Currently, three approaches are recommended by EPA to assess the presence of bioconcentratable or bioaccumulative substances (not covered by water quality criteria) in surface waters and effluents. These are the tissue residue measurement, effluent measurement, and sediment assessment options. The draft EPA guidance document (Burkhard *et al.*, 1991) on the "Assessment and Control of Bioconcentratable Contaminants in Surface Water" describes in detail the rationale, experimental designs and analytical methods required for these approaches. In this primer, only the salient features of these methods are covered, and an evaluation of each one is provided. Also, the use of transplanted bivalve molluscs and the lipid-containing SPMDs are examined briefly as potential options for the determination of bioaccumulative chemicals.

TISSUE RESIDUE OPTION

The tissue residue approach involves measurement of the concentrations of contaminants in tissue samples of indigenous organisms. More specifically it involves the collection of fish and/or bivalves, extraction and enrichment of organic contaminants present in tissues, and analysis of these purified extracts with gas chromatography-mass spectrometry (GC-MS), which is generally the most definitive (i.e., structural identification) analytical instrument for bioconcentratable compounds.

This approach relies on the comparison of tissue concentrations of organisms in receiving water sites to those in similar organisms collected from relatively uncontaminated reference or control sites. Comparisons can also be made to regulatory tissue residue values, if available. For successful implementation of the tissue analysis option, samples must be obtained that are representative of the organisms in the receiving water and they must be analyzed in accordance with good laboratory practices.

Method Evaluation

Although the tissue residue measurement approach is environmentally realistic and has been the method of choice, it has problems because the same or similar species may not be collected at the test and reference/control sites, making comparisons difficult. Also, field surveys are often the most expensive approach due to collection and tissue analysis costs. Finally, even with the successful collection of bivalve molluscs, apparent species differences in the ability of these organisms to bioaccumulate contaminants limit the certainty of this approach.

EFFLUENT OPTION

The effluent option allows measurement of the concentrations of organic bioconcentratable chemicals in effluent samples. This approach involves the collection of effluent samples, the extraction of the organic chemicals from them, and the separation and analysis of the bioconcentratable chemicals from the other chemical components of the effluent sample. The effluent procedure is designed to sort the results of the initial screening analysis in order to determine which of the contaminants pose a hazard.

The BCFs of compounds identified in the effluent that appear to pose a hazard are estimated from log K_{ow} - log BCF relationships discussed earlier or compared to measured values of BCF that followed ASTM's "Standard Practice for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Molluscs, 1022-84." Obviously, the likelihood of the existence of experimental data of this type is very low.

Method Evaluation

The effluent assessment option will not detect all bioconcentratable chemicals that may be present in aquatic organisms. The analytical procedures outlined in this approach will detect only acid-stable (most PAHs are not stable under the strong acid-treatment conditions used) nonpolar organic chemicals with log K_{ow} values of 3.5 and greater that

can be successfully analyzed using GC-MS. Also, generation of artifacts from the acid treatment can reduce the certainty that compounds identified were present in the effluent. These procedures are fairly robust for chlorinated hydrocarbons. Some of the limitations include analytical interferences from hydrocarbons sometimes present in refinery effluents, lack of sensitivity of the analytical method, and the probable lack of reliably measured BCFs and/or BAFs for detected bioaccumulative substances.

SEDIMENT ASSESSMENT OPTION

In some receiving waters, sediments may be a significant source of bioaccumulative chemicals. Analysis of sediments for bioaccumulative contaminants can determine the identity and concentrations of pollutants in sediment samples subjected to contamination from different sources. Since sediments can accumulate these types of pollutants over relatively long periods of time and can be preferential sorption sites, the bioaccumulative chemicals are generally present in greater concentrations in sediments than in a given effluent sample. In some cases, this may facilitate detection of contaminants which are present in an effluent or other sources at very low concentrations or are only released periodically. Data from sediment evaluations may also be used to estimate the spatial extent of a contaminated area, monitor the benefits derived from remediation activities, and aid in locating sources.

Method Evaluation

Similar to the effluent option, the sediment assessment option as described by EPA is useful only for acid-stable compounds with log K_{ow} values greater than 3.5. Potential interferences in sediment samples that can reduce analytical sensitivity and preclude analysis include high levels of hydrocarbons and elemental sulfur. Also, lack of reliable K_{oc} and BCF values for the bioaccumulative substances detected in the sediment analysis may prevent accurate assessment of the bioaccumulation potential. However, unlike organisms, it should be possible to detect a broader spectrum of potentially bioconcentratable chemicals because losses of chemicals from sediment are largely mediated by thermodynamic, passive partitioning processes, and not by metabolism or active export.

OVERVIEW OF EPA ASSESSMENT METHODS

In 1992, fifteen laboratories participated in a round-robin study on the three options proposed by EPA (Burkhard *et al.*, 1991) for assessment of bioconcentratable contaminants (not covered by water quality criteria) in effluents and receiving waters. Wong *et al.* (1997) evaluated the data resulting from the participation of five (three industrial and two contract laboratories) of the fifteen laboratories in the round-robin study. Small changes in analytical procedures were made (as necessary) in the EPA proposed methods. Wong *et al.* (1997) reported that analytical variance explains a large portion of the observed intralaboratory variability in surrogate recoveries. Interlaboratory variability in surrogate recovery was also large. All five laboratories isolated and positively identified only three of eight spiked compounds at greater than 50% frequency, using the EPA proposed analytical procedures and the GC-MS library search method. The inability of the MS library search to detect some of the spiked reference chemicals reflects the low prospect that the method will permit detection of unknown bioconcentratable chemicals. In general, the proposed multi-step procedures for each matrix were found to be complex, prone to loss of some of the chemicals of interest, and time consuming.

OTHER POTENTIAL APPROACHES

The results of the aforementioned round-robin study (Wong *et al.*, 1997) and the discussion on existing approaches in this document suggest the need for additional methods to assess bioaccumulative chemicals in water.

Transplanted Sentinel Organisms

This is essentially the tissue residue option with several differences. In this case the species of organism (i.e., bivalve or fish) can be carefully selected for minimal MFO activity (i.e., low biotransformation rates of chemicals) and for survival in different water qualities and contaminated environments. Also the issue of organism nonavailability at appropriate sites in the receiving waters can be circumvented by transplanting moored arrays of bivalves or using caged fish (Farrington *et al.*, 1987).

Unfortunately, the availability of organisms with very low contaminant backgrounds for transplants is problematic, and the organisms chosen sometimes require extensive depuration periods before use. Also, a variety of factors are known to influence the relationship between the presence of a chemical in an effluent and its bioaccumulation in bivalves and other fish tissues. These include reproductive status, nutritional status, temperature, salinity, particulate concentration in the water column, environmental turbulence and chemical stressors. Generally speaking, animal health and survival in hostile environments are the major difficulties with this approach. Also, residues associated with the gut contents of bivalves are often measured along with those actually incorporated into tissues, positively biasing reported concentrations. Finally, the costs of this type of study can be even greater than those of the other EPA proposed options.

Semipermeable Membrane Device Technology

The lipid-containing SPMD (Figure 2-10, page 3-6) is a relatively new technology designed to sample bioavailable lipophilic contaminants from water, air, and sediments (Huckins *et al.*, 1990; Huckins *et al.*, 1993). It is constructed from a layflat polyethylene membrane having permeability characteristics similar to fish gills (Huckins *et al.*, 1996; Lieb and Stein, 1969) with a thin film of triolein (a major fat or lipid found in most organisms) sealed inside. The device is designed to mimic the bioconcentration (uptake from water) of chemicals by organisms without the many variables associated with the use of live animals. SPMDs are made of materials that are reproducible, and the uptake and elimination of chemicals is controlled by readily modeled passive partitioning (thermodynamic) processes. The current status of this technology has been reviewed in a book chapter by Huckins *et al.* (1996) and the use of these devices in aquatic systems is becoming widespread. SPMDs are now commercially available (Environmental Sampling Technologies, St. Joseph, MO).

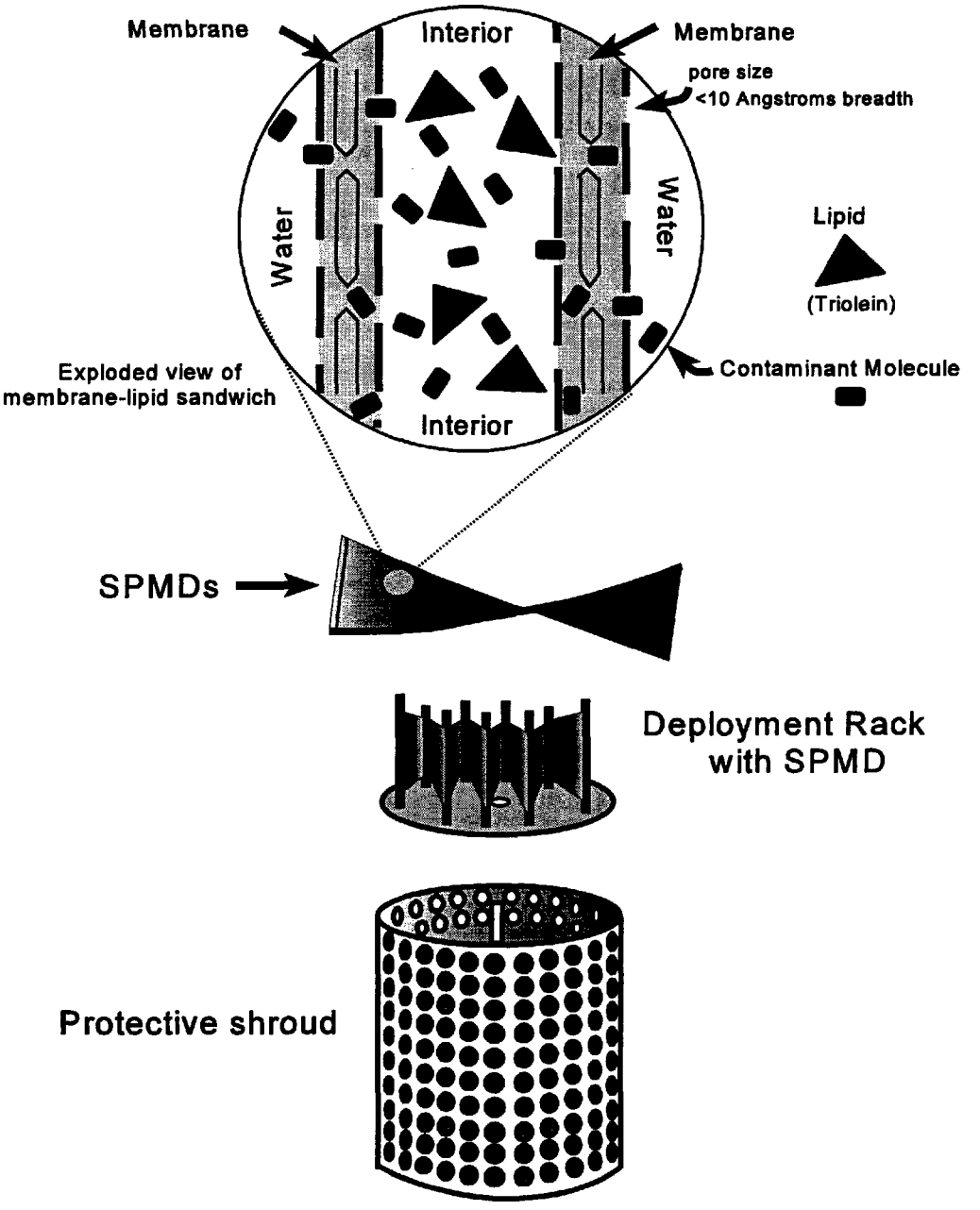

Figure 2-10. The lipid containing semipermeable membrane device (SPMD) and a typical deployment apparatus.

Conceptually, SPMDs represent a bridge between the tissue residue option and the effluent option for assessing bioaccumulative substances in effluents and receiving waters. SPMDs sample water, as does the effluent extraction option, but they passively extract a portion of the effluent or receiving water (*in situ*) for a period of days-to-weeks. They only sequester chemicals that are dissolved (freely bioavailable, not particulate-bound). The magnitude of the equilibrium partition coefficients of lipophilic

contaminants between the triolein lipid used in SPMDs and water has been shown to be nearly identical to that observed for octanol and water (i.e., K_{ow}), which in turn is correlated to tissue BCF. Thus, the same partitioning phenomenon that leads to concentration of most lipophilic chemicals in aquatic organisms (analyzed in the tissue residue option) is the driving force for residue accumulation in SPMDs. At equilibrium, the concentration factor (CF) of a persistent nonmetabolized chemical in SPMD triolein ($SPMD_L$ CF) should approximate the lipid-based BCF of the chemical in tissues (BCF_L), i.e.,

$$SPMD_L \text{ CF} \sim BCF_L \sim K_{ow} \qquad \text{(Equation 3-1)}$$

Because SPMDs have high lipid content and only eliminate chemicals by slow, passive dissipation, they are best suited to sample chemicals during the long linear portion (generally weeks) of contaminant uptake, and thus equilibrium is seldom achieved.

The same modeling approach applied to the uptake of chemicals by organisms discussed in "organism-related factors" can be applied to SPMD uptake. SPMD uptake rates (k_1) are given in liters of water extracted per day by one gram of SPMD or triolein, and the units are the same as k_1 for organisms. SPMDs have also been shown to have similar uptake rates (k_1) as fish and bivalves (Prest *et al.*, 1992; Gale *et al.*, 1996) and range from about 200 to 7,000 mL/d x g for priority pollutant PAHs (Petty *et al.*, 1994); note similarity to invertebrate k_1 data presented earlier. Unlike living organisms, the reproducibility of SPMD sampling in different environments (SPMDs are generally unaffected by water quality other than temperature) has allowed estimates (Huckins *et al.*, 1996; Ellis *et al.*, 1995) of water concentrations to be made with two-fold accuracy, which enable calculations of the time-weighted flux of dissolved chemicals in aquatic systems.

Data on the SPMD sampling rates of the priority pollutant PAHs are now available in a report to the National Fish and Wildlife Foundation (Petty *et al.*, 1994) which permit

calculation of average PAH water concentrations in effluents from SPMD concentrations. In general, the volume of water sampled by a 1 gram triolein SPMD in exposures greater than 2 weeks far exceeds the 12 liters collected and extracted in the effluent option. When unknown chemicals are sequestered in SPMDs, extracts or dialysates of SPMDs can be analyzed similarly to water samples from the "effluent option" (note that acid treatment of SPMD extracts is unnecessary). However, cleanup of SPMD extracts for analysis of unknowns is less problematic than cleanup of animal tissues, and may well be easier than that of large-volume water extracts from the "effluent option." In general, a compound's log K_{ow} should be greater than 3.5 to be adequately concentrated by SPMDs (also applicable to the effluent option).

SPMDs do not provide data on the potential for a compound to biomagnify up the food chain, nor do they provide estimates of the contribution of dietary uptake, which is also the case for the previously discussed effluent and sediment assessment options. However, the major route of uptake of PAHs and other hydrocarbons by aquatic organisms appears to be via water (Connell, 1990; Pruell *et al.*, 1986). Thus, factors (dietary and food chain) that cause BAFs in some predators to be greatly elevated above the BCF (water route only) are probably not a concern for PAHs and other petroleum-related chemicals. Biofouling does reduce the rate of chemical uptake by SPMDs. However, this problem can be corrected for by use of a permeability reference compound as discussed by Huckins *et al.* (1996). SPMDs are unique in that they provide truly dissolved concentrations of chemicals (Ellis *et al.*, 1995). Water concentration data from the use of SPMDs may ultimately be directly applicable to "water quality criteria." Finally, the cost of SPMD sampling and analysis appears to be less than the use of transplanted or endemic organisms.

REFERENCES

Barron, M.G. 1990. Bioconcentration. *Environmental Science and Technology.* 24: pp. 1612-1618.

Boese, B.L., H. Winsor, H. Lee II, S. Echols, J. Pelletier, and R. Randall. 1996 (manuscript). Steady-State Accumulation Factors (AF's) for 13 PCB Congeners and Hexachlorobenzene in (*Macoma nasuta)* Exposed to Sediments of Variable Total Organic Carbon Content.

Boese, B.L. and H. Lee II. 1992. *Synthesis of Methods to Predict Bioaccumulation of Sediment Pollutants.* Environmental Research Laboratory - Narragansett, Report Number 232. U.S. Environmental Protection Agency, Newport, OR.

Brown, R.A. 1978. *Fate and Effects of Polynuclear Aromatic Hydrocarbons in the Aquatic Environment.* API Publication No. 4297. American Petroleum Institute, Washington, D.C.

Bruggeman, W.A., A. Opperhuizen, O. Hutzinger. 1984. Bioaccumulation of Super-lipophilic Chemicals in Fish. *Toxicology and Environmental Chemistry.* 7: pp. 173-189.

Burkhard, L.P., J.R. Cannell, K.W. Dowell, W.J. Morrow, B.R. Sheedy and R. Brondes. 1991. *Assessment and Control of Bioconcentratable Contaminants in Surface Waters.* Draft guidance document. U.S. Environmental Protection Agency, Office of Water, Washington, D.C. 20460.

Connell, D.W. 1990. *Bioaccumulation of Xenobiotic Compounds.* CRC Press Inc., Boca Raton, FL.

Connolly, J.P. and C.J. Pedersen. 1988. A Thermodynamic-Based Evaluation of Organic Chemical Accumulation in Aquatic Organisms. *Environmental Science and Technology.* 22: pp. 99-103.

Derr, S.K., M.J. Zabik. 1974. Bioactive Compounds in the Aquatic Environment: Studies on the Mode of Uptake of DDE by the Aquatic Midge, *(Chironomus tentans) (Diptera chironomidae). Archives of Environmental Contamination and Toxicology.* 2: pp. 152-164.

Ekelund, R. 1989. Bioaccumulation and Biomagnification of Hydrophobic Persistent Compounds as Exemplified by Hexachlorobenzene. In L. Landner, ed. *Chemicals in the Aquatic Environment.* Springer-Verlag, Berlin, Germany. pp. 128-149.

Ellis, G.S., J.N. Huckins, C.E. Rostad, C.J. Schmitt, J.D. Petty and P. MacCarthy. 1995. Evaluation of Lipid-Containing Semipermeable Membrane Devices (SPMDs) for Monitoring Organochlorine Contaminants in the Upper Mississippi River. *Environmental Toxicolology and Chemistry.* 14: pp. 1875-1884.

Farrington, J.W., A.C. Davis, B.W. Tripp, D.K. Phelps and W.B. Galloway. 1987. "Mussel Watch" - Measurements of Chemical Pollutants in Bivalves as One Indicator of Coastal Environmental Quality. In T.P. Boyle, ed. *New Approaches to Monitoring Aquatic Ecosystems.* ASTM Special Technical Publication 940. ASTM 1916 Race Street, Philadelphia, PA. pp. 125-139.

Ferraro, S.P., H. Lee II, R. J. Ozretich, and D.T. Specht. 1990. Predicting Bioaccumulation Potential: a Test of a Fugacity Based Model. *Archives of Environmental Contamination and Toxicology.* 19: pp. 386-394.

Gale, R.W., J.N. Huckins, J.C. Meadows, P.H. Peterman, J.D. Petty, D.E. Tillitt, and L. Williams. 1996. Comparison of the Uptake of Dioxin-Like Compounds by Caged Channel Catfish and Semipermeable Membrane Devices in the Saginaw River, Michigan. *Environmental Science and Technology.* In press.

Guiney, P.D., M.J. Melancon, Jr., J.J. Lech, R.E. Peterson. 1979. Effects of Egg and Sperm Maturation and Spawning on the Distribution and Elimination of a Polychlorinated Biphenyl in Rainbow Trout (*Salmo gairdneri*). *Toxicology and Applied Pharmacology.* 47: pp. 261-272.

Hansch, C. and A. Leo. 1995. *Exploring QSAR, Fundamentals and Applications in Chemistry and Biology.* ACS Professional Reference Book, American Chemical Society, Washington, D.C.

Hayton, W.L. and M.G. Barron. 1990. Rate-limiting Barriers to Xenobiotic Uptake by the Gill. *Environmental Toxicolology and Chemistry.* 9: pp. 151-157.

Huckins, J.N. G.K. Manuweera, J.D. Petty, D. Mackay and J.A. Lebo. 1993. Lipid-Containing Semipermeable Membrane Devices for Monitoring Organic Contaminants in Water. *Environmental Science and Technology.* 27: pp. 2489-2496.

Huckins, J.N., J.D. Petty, J.A. Lebo, C.E. Orazio, H.F. Prest, D.E. Tillitt, G.S. Ellis, B.T. Johnson and G.K Manuweera. 1996. Semipermeable Membrane Devices (SPMDs) for the Concentration and Assessment of Bioavailable Organic Contaminants in Aquatic Environments. In G.K. Ostrander, ed. *Techniques in Aquatic Toxicology.* Lewis Publishers, Boca Raton, FL.

Huckins, J.N., M.W. Tubergen and G.K. Manuweera. 1990. Semipermeable Membrane Devices Containing Model Lipid: a New Approach to Monitoring the Bioavailability of Lipophilic Contaminants and Estimating Their Bioconcentration Potential. *Chemosphere.* 20: pp. 533-552.

Kidd, K.A., D.W. Schindler, D.C. Muir, W.L. Lockhart and R.H. Hesslein. 1995. High Concentrations of Toxaphene in Fishes from a Subarctic Lake. *Science.* 269: pp. 240-242.

Lee II, H. 1992. Models, Muddles, and Mud: Methods to Predict Bioaccumulation from Sediment. In G.A. Burton, ed. *Contaminated Sediment Toxicity Assessment.* Lewis Publishers, Chelsea, MI. pp. 267-293.

Lee, R.F., W.S. Gardner, J.W. Anderson, J.W. Blaylock, and J. Barwell-Clarke. 1978. Fate of Polycyclic Aromatic Hydrocarbons in Controlled Ecosystem Enclosures. *Environmental Science and Technology.* 12: pp. 832-838.

Lehr, R.E., S. Kumer, W. Levin, A.W. Wood, R.L. Chang, A.H. Conney, H. Yagi, J.M. Sayer and D.M Jerina. 1985. The Bay Region Theory of Polycyclic Aromatic Hydrocarbon Carcinogenesis. In R.G. Harvey, ed. *Polycyclic Hydrocarbons and Carcinogenesis.* American Chemical Society, Washington, D.C. pp.63-84.

Lieb, W.R. and W.D. Stein. 1969. Biological Membranes Behave as Non-Porous Polymeric Sheets with Respect to the Diffusion of Non-Electrolytes. *Nature.* 224: pp. 240-243.

Mackay, D. 1994. Unraveling the Choreography of Contaminant Kinetics: Approaches to Quantifying the Uptake of Chemicals by Organisms. In J.L. Hamelink, P.F. Landrum, H.L. Bergman and W.H. Benson, eds. *Bioavailability: Physical, Chemical and Biological Interactions.* Lewis Publishers, Chelsa, MI. pp. 171-177.

Mackay, D., W.Y. Shiu and K.C. Ma. 1992. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals*, Volume II. Lewis Publishers, Chelsea, MI.

McKenyan, O.G., G.T. Ankley, G.D. Veith and D.J. Call. 1994. QSARs for Photoinduced Toxicity: 1. Acute Lethality of Polycyclic Aromatic Hydrocarbons to (*Daphnia magna*). *Chemosphere.* 28: pp. 567-582.

McKim, J., P. Schmieder, and G. Veith. 1985. Absorption Dynamics of Organic Chemical Transport Across Trout Gills as Related to Octanol-Water Partition Coefficient. *Toxicology and Applied Pharmacology.* 77: pp.1-10.

Neff, J.M. 1985. Polycyclic Aromatic Hydrocarbons. In G.M. Rand, and S.R. Petrocelli, eds. *Fundamentals of Aquatic Toxicology*. Hemisphere Publishing Corporation, Washington, D.C. pp. 416-454.

Opperhuizen, A., E.W. Velde, F.A.P.C. Gobas, D.A.K. Liem, J.M.D. Steen, and O. Hutzinger. 1985. Relationship Between Bioconcentration in Fish and Steric Factors of Hydrophobic Chemicals. *Chemosphere*. 14: pp. 1871.

Petty, J.D., J.N. Huckins, C.E. Orazio, J.A. Lebo, R.C. Clark, and V.L. Gibson. 1994. *Laboratory Studies of the Use of Semipermeable Membrane Devices (SPMDs) as Passive Water Samplers of Polyaromatic Hydrocarbons (PAH) Priority Pollutants.* Draft report to American Petroleum Institute. Midwest Science Center, USDI.

Prest, H.F., W.M. Jarman, S.A. Burns, T. Weismüller, M. Martin and J.N. Huckins. 1992. Passive Water Sampling via Semipermeable Membrane Devices (SPMDs) in Concert with Bivalves in the Sacramento/San Joaquin River Delta. *Chemosphere*. 25: pp. 1811-1823.

Pruell, R.J., J.L. Lake, W.R. Davis, and J.G. Quinn. 1986. Uptake and Depuration of Organic Contaminants by Blue Mussels (*Mytilus edulis*) Exposed to Environmentally Contaminated Sediment. *Marine Biology*. 91: pp. 497-507.

Rubinstein, N.I., R.J. Pruell, B. Taplin, J.A. LiVolsi, and C.B. Norwood 1990. Bioavailability of 2,3,7,8-TCDD, 2,3,7,8-TCDF and PCBs to Marine Benthos from Passaic River Sediments. *Chemosphere*. 20: pp. 1097-1102.

Southworth, G.R., J.J. Beauchamp, and P.K. Schmieder. 1978. Bioaccumulation Potential of Polycyclic Aromatic Hydrocarbons in *(Daphnia pulex)*. *Water Research*. 12: pp. 973-977.

Spacie, A. and J.L. Hamelink. 1992. Alternative Models for Describing the Bioconcentration of Organics in Fish. *Environmental Toxicolology and Chemistry*. 1: pp.309-320.

Spacie, A. and J.L. Hamelink. 1985. Bioaccumulation. In G.M. Rand, and S.R. Petrocelli, eds. *Fundamentals of Aquatic Toxicology*. Hemisphere Publishing Corporation, Washington, D.C. pp. 495-525.

Thomann, R.V., J.P. Connolly, T.F. and Parkerton. 1992. Modeling Accumulation of Organic Chemicals in Aquatic Food Webs. In F.A. Gobas, P.C. and McCorquodale, J.A., Eds. *Chemical Dynamics in Freshwater Ecosystems*. Lewis Publishers, Boca Raton, Fl. pp. 153-185.

Veith, G.D., O.G. Mekenyan, G.T. Ankley and D. J. Call. 1996. A QSAR Analysis of Substituent Effects on the Photo-Induced Acute Toxicity of PAHs. *Environmental Toxicolology and Chemistry.* In press.

Wong, D.C.L., R. van Compernolle, E.Y. Chai, R.D. Fitzpatrick and W.J. Bover. 1997. A Multilaboratory Evaluation of Analytical Methods for Estimating Bioconcentratable Contaminants in Effluents, Tissues and Sediments. *Environmental Toxicolology and Chemistry.* 16(4):617-624.

Zitko, V. and O. Hutzinger. 1976. Uptake of Chloro- and Bromobiphenyls, Hexachloro- and Hexa-bromobenzene by Fish. *Bulletin of Environmental Contamination and Toxicology.* 16: pp. 665-673.

GLOSSARY

Absorption	The process by which molecules of a chemical move into or dissolve into another matrix or compartment.
Assimilation	The fractional amount of a chemical taken up or absorbed by an organism's skin, gills, or gut relative to the amount of the chemical present in the contiguous exposure medium; for example, gastrointestinal assimilation efficiency for PCBs in ingested fish food (excludes sediments) ranges from about 0.5 to 0.85 or 50 to 85%.
Bioaccumulation	Uptake and retention of a substance by an organism from the surrounding media (e.g., water column, pore water, sediment/soil, and air), from food, and in some cases ingested particulates; bioaccumulated residues are referred to as the body burden (strictly speaking, excludes gut contents) and represent the contribution of all sources.
Bioaccumulation factor (BAF)	Measure of a chemical's tendency to bioaccumulate. Often used interchangeably with bioconcentration factor (BAF) in the literature. BAF is generally equal to or greater than BCF (defined below) and can be much higher for predatory fishes and fish-eating animals.
Bioavailablity	There are two definitions of chemical bioavailability, depending on specificity. Toxicological bioavailability refers to the fraction of the total dose of chemical taken up or absorbed by an organism that arrives at the site of toxic action. Environmental bioavailability refers to the fraction of a chemical in an exposure medium (e.g., water) that is available for uptake by organisms. Note that a compound can be environmentally bioavailable but not significantly bioaccumulated because of its high depuration rate from organisms.
Bioconcentration	Uptake of a substance by an organism from the surrounding medium through respiratory membranes (e.g., gills) or other external body surfaces. Dietary input is not included.
Bioconcentration factor (BCF)	The measure of a chemical's tendency to bioconcentrate. The BCF is calculated at steady state by dividing the concentration of the chemical in the exposed organism's

	tissues by the concentration of the chemical in the exposure medium. If the BCF of a chemical is one or less than one, then bioconcentration does not occur.
Biomagnification	The process by which the concentration of a compound increases in different organisms, occupying successively higher trophic levels.
Biotransformation (Biodegradation)	A biologically catalyzed conversion of a chemical into another. Often used interchangeably with metabolism and biodegradation.
Compartment	A distinct component or phase, such as biota, sediment and water, in the environment that participates in chemical exchange processes. Also it can refer to organs or a hypothetical medium within an organism with chemical exchange kinetics that are different from the rest of the organism.
Degradation	A change in the structure of a parent molecule, i.e., the loss and/or gain of an atom or a group of atoms as well as the accompanied changes in the affected chemical bonds. Inclusive of both physical-chemical and biological processes. The extent of change in the parent molecules ranges from partial to the complete breakdown into inorganic molecules, e.g., water and carbon dioxide.
Depuration	The elimination of a chemical from an organism by exchange processes that include outward diffusion across gills, excretion via waste elimination, and/or metabolism. Used interchangeably with clearance and elimination.
Diffusion	The process whereby molecules spontaneously move from regions of higher concentration to regions of lower concentration.
Dissolved organic carbon (DOC)	The organic carbon present in waterborne compounds [i.e., natural polymers such as humics, and particulates having diameters less than 0.1 micrometers (1×10^{-7}m)]. Strictly speaking, DOC represents only the carbon in molecules that are truly dissolved.

Equilibrium	A state of balance between the concentrations of a chemical in two interactive components, phases, or compartments (e.g., fish and water) which, strictly speaking, is limited to mass-balance systems with only passive-diffusive exchange processes.
Food chain	A series of living organisms of succeedingly higher trophic levels with each level feeding upon the next lower level.
Fractional-organic-carbon (f.o.c.)	The fraction (expressed as % except when used to derive K_{oc}) of the total mass of a sediment that is organic carbon. Typically sediments range from 0.5 to 10% f.o.c.
Functional group (chemical)	An atom or group of atoms in a molecule that largely determines its properties and defines its structural family (i.e., the class of compounds to which it belongs). When a molecule contains multiple functional groups, its overall properties will generally reflect a composite of the properties of the individual functional groups.
Hydrophobic	Chemicals with low water solubility are hydrophobic (water-hating). Compounds that are nonpolar, i.e., lacking polar functional groups.
Lipid (fat)	Biochemical substances in organisms that are soluble in nonpolar organic solvents (generally only sparingly soluble in water) and constitute a major storage and structural component of living cells; includes fats, waxes and other related compounds.
Lipophilic	Chemicals with a high lipid solubility are lipophilic (lipid-loving). Nonpolar or hydrophobic compounds are generally lipophilic.
Natural sink	Any natural material, which includes certain clay minerals and the organic carbon of sediments and soils, that has a high affinity for contaminants. Note that a natural sink is not a permanent repository for chemicals as some losses of residues always occur; e.g., highly contaminated sediments often act as sources for low level contaminants.
Nonpolar (chemical)	A substance that normally will not disassociate into ions (atoms or groups of atoms with electrical charges); a

	nonelectrolyte or neutral molecule; chemicals whose molecular structures lack functional groups having strong interactions (such as hydrogen bonding) with other molecules.
Octanol-water partition coefficient	The equilibrium ratio of the concentration of a chemical in n-octanol relative to its concentration in water. For example, a K_{ow} (K_{ow} or P) of 100,000 means that at equilibrium, the concentration of a chemical will be 100,000 times greater in octanol than in water (equivalent volumes of octanol and water). Note that prior to K_{ow} determination, the two phases are agitated while in intimate contact. The K_{ow} is the most accepted laboratory test (surrogate) for studying the relationship between chemical partitioning in an organism's lipids and water, and the potential for biological effects.
Organic carbon (sediment)-water partition coefficient (K_{oc}; $K_{oc} = K_p/f.o.c.$)	The equilibrium or steady state distribution coefficient derived from dividing the concentration of a chemical in the organic carbon portion or fraction (f.o.c.) of a sediment by its concentration in water.
Organic compound	A chemical resulting from the union of separate elements, one of which is carbon; includes both biological and anthropogenic origins.
Particulate organic carbon (POC)	Organic carbon in suspended and bed sediment particles having diameters greater than 0.1 micrometers. Generally, inorganic minerals represent the largest fraction of the total particle mass.
Partition coefficient	Distribution of a chemical between two immiscible phases, generally measured at equilibrium.
Polycyclic aromatic hydrocarbons (PAHs)	PAHs are a class of organic compounds characterized by carbon and hydrogen atoms in the form of two or more fused aromatic (benzene) rings. Two aromatic rings are fused when a pair of carbon atoms is shared. The resulting structure generally lies in a single plane or is flat.
Priority Pollutant PAHs	Sixteen PAHs chosen by the U.S. Environmental Protection Agency (EPA) as PAHs representative of hazards posed by this class of chemicals. They are the following:

	acenaphthene, acenaphthylene, anthracene, benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-c,d]pyrene, naphthalene, phenanthrene, and pyrene.
Rate constant (k)	A mathematical proportionality constant that relates the rate of change in a process (e.g., chemical uptake, elimination, metabolism, etc.) to the concentration of the chemical undergoing the change. Uptake rate constants are generally designated as k_1 and depuration rate constants as k_2.
Sediment-water partition coefficient (K_p)	The equilibrium or steady state distribution coefficient derived from dividing the concentration of a chemical in sediment by the chemical concentration in water.
Sorption	The process by which molecules of a chemical dissolve into or are retained on the surface of a material. Inclusive of absorption and adsorption processes.
Steady state	A state of balance between the concentrations of a chemical in two interactive components such as fish and water, that includes both passive (e.g., diffusion) and active (e.g., ingestion of food with chemical residues, egestion, etc.) exchange processes. State at which the mass of chemical input is equal to the mass of chemical output. Steady state concentrations in organisms can be higher (e.g., air breathing and fish-eating aquatic animals and birds can ingest large quantities of contaminated food but lack efficient mechanisms of depuration such as an organism-to-water exchange interface), equal to or less than (e.g., parent compound is metabolized and breakdown products are depurated) equilibrium values.
Trophic level	One of the successive levels of a pyramidal food web, or food chain; primary producers (e.g., phytoplankton) constitute the lowest trophic level, and dominant carnivores constitute the highest trophic level.

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