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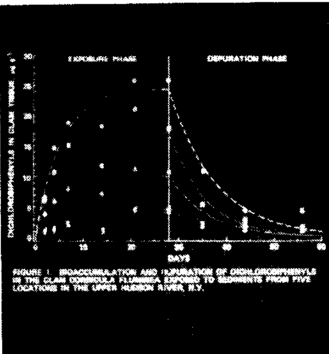

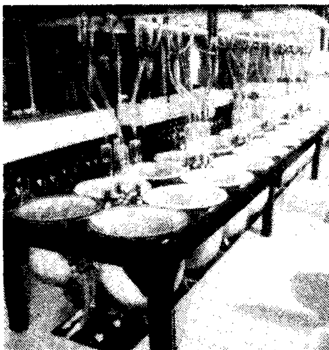

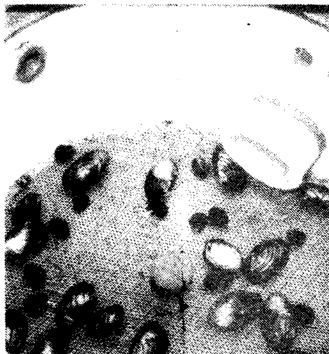

LONG-TERM EFFECTS OF DREDGING OPERATIONS PROGRAM

MISCELLANEOUS PAPER D-91-2

ASSESSING BIOACCUMULATION IN AQUATIC ORGANISMS EXPOSED TO CONTAMINATED SEDIMENTS

by

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July 1991

Final Report

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This paper synthesizes previous work on bioaccumulation to provide a working document for the environmental assessment of impacts on the aquatic environment due to bioaccumulation of sediment contaminants resulting from dredging operations and dredged material placement. Emphasis is placed on explanation of basic concepts concerning, and factors influencing, sediment contaminant bioaccumulation and bioavailability. The paper presents several numerical methods for assessing bioaccumulation, including a simple method for estimating theoretical bioaccumulation potential (TBP) from sediment chemistry for neutral organic chemicals. Methods are also given for projecting contaminant concentrations in organism tissues when steady state is achieved, based on laboratory or field exposures to contaminated sediments. These assessments are presented in the context of the US Environmental Protection Agency's tiered testing approach for dredged material evaluation. The various numerical methods for bioaccumulation assessment are illustrated and compared using step-by-step example calculations with hypothetical and actual data.

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Bioaccumulation
Bioavailability
Contaminants
Kinetic
Neutral organic chemicals
Partition coefficients
Preference factor
Sediment
Steady state
TBP
Thermodynamic

PREFACE

The research on which this paper is based was conducted by the US Army Engineer Waterways Experiment Station (WES), Environmental Laboratory (EL), Vicksburg, MS. Funding was provided by the Long-Term Effects of Dredging Operations (LEDO) Program, Work Unit 31772. The LEDO Program is sponsored by Headquarters, US Army Corps of Engineers and is managed within the Environmental Effects of Dredging Programs, Dr. Robert M. Engler, Manager and LEDO Coordinator. The Technical Monitor was Mr. Joe Wilson.

Authors of this paper were Ms. Joan U. Clarke and Mr. Victor A. McFarland of the Contaminant Mobility and Regulatory Criteria Group (CMRCG), Ecosystem Research and Simulation Division (ERSD), EL. The paper was edited by Ms. Janean Shirley of the WES Information Technology Laboratory.

Principal Investigator was Mr. Victor A. McFarland, leader of the Aquatic Contaminants Team, CMRCG. The study was conducted under the general supervision of Dr. Lloyd H. Saunders, Chief, CMRCG, and Mr. Donald L. Robey, Chief, ERSD. Chief of EL was Dr. John Harrison.

Chemical analyses of polychlorinated biphenyls and lipids for Hudson River bioaccumulation data included in this paper were done by Dr. Joseph M. O'Connor, New York University Medical Center, Institute of Environmental Medicine, Tuxedo, NY. The authors thank Drs. David Moore, Judith Pennington, and Thomas Wright, EL, and Mr. Francis J. Reilly, Jr., AScI Corporation, for technical review of the paper.

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CONTENTS

Page

PREFACE 1

PART I: INTRODUCTION 3

PART II: BASIC CONCEPTS 5

Definitions 5
Factors Influencing Bioaccumulation 5
Kinetics of Uptake and Elimination 33

PART III: ASSESSMENT OF BIOACCUMULATION 35

Environmental Assessment of Sediments 35
The Tiered Testing Approach 35
Bioaccumulation Potential (Tier II) 37
Bioaccumulation Testing (Tiers III and IV) 52
Example Calculations for Each Method of Bioaccumulation Assessment 60

REFERENCES 70

APPENDIX A: NOTATION A1

APPENDIX B: SUMMARY OF EQUATIONS B1

APPENDIX C: SAS PROGRAM STATEMENTS FOR PLOTTING BIOACCUMULATION DATA AND FITTED REGRESSION CURVES C1

ASSESSING BIOACCUMULATION IN AQUATIC ORGANISMS EXPOSED TO CONTAMINATED SEDIMENTS

PART I: INTRODUCTION

1. The US Army Corps of Engineers Long-Term Effects of Dredging Operations (LEDO) Program was established in the early 1980's to develop and improve methods for predicting long-term environmental consequences of dredging operations and for minimizing any adverse impacts of dredged material placement (Engler, Patin, and Theriot 1990). The Bioaccumulation Work Unit ("Toxic Substances Bioaccumulation in Aquatic Organisms") of LEDO addresses the fundamental processes involved, and develops techniques for prediction and assessment of toxic chemical bioaccumulation in aquatic organisms exposed to contaminated sediments. This paper represents a culmination and synthesis of work conducted to date under the Bioaccumulation Work Unit. As such, the paper draws heavily on information in the following publications completed under LEDO:

"Activity-Based Evaluation of Potential Bioaccumulation From Sediments" (McFarland 1984)

"Testing Bioavailability of Polychlorinated Biphenyls from Sediments Using a Two-Level Approach" (McFarland and Clarke 1986)

"Simplified Approach for Evaluating Bioavailability of Neutral Organic Chemicals in Sediment" (McFarland and Clarke 1987)

"Evaluating Bioavailability of Neutral Organic Chemicals in Sediments--A Confined Disposal Facility Case Study" (Clarke, McFarland, and Dorkin 1988)

"Influence of Environmental Variables on Bioaccumulation of Mercury" (Clarke, Lutz, and McFarland 1988)

"Factors Influencing Bioaccumulation of Sediment-Associated Contaminants by Aquatic Organisms" (McFarland, Lutz, and Reilly 1989).

The publications listed above form the core of this paper and will not be cited routinely herein. Pertinent bioaccumulation investigations performed outside of LEDO and not referenced in the works listed above will be cited.

2. The purpose of this paper is to provide a working document for Corps regulators and others involved in the environmental assessment of impacts on the aquatic environment from dredging operations and dredged material placement. The paper should facilitate an understanding of the basic concepts concerning, and factors influencing, sediment contaminant bioaccumulation and

bioavailability. How bioaccumulation assessments fit into the tiered testing approach for dredged material evaluation (US Environmental Protection Agency 1990) is explained. The paper also provides the derivation, step-by-step procedures, and example applications of a simple method for estimating theoretical bioaccumulation potential (TBP) for neutral organic contaminants. Finally, methods are given for projecting contaminant concentrations in organism tissues when steady state is achieved, and for calculating a numerical measure of contaminant bioavailability.

3. Newly defined terms, and topic headings within subsections are presented in boldface type in the text. SAS program statements for plotting bioaccumulation data and fitted regression curves are presented in Appendix A. For convenience, equations are summarized in Appendix B and symbols and abbreviations are listed in the Notation (Appendix C).

PART II: BASIC CONCEPTS

Definitions

4. Chemicals can move through the aquatic environment by various sorption processes. **Adsorption** refers to the attachment (binding) of a chemical to the exterior of a substrate, as in the binding of trace metals or organic chemicals to sediment particles. **Absorption** refers to the uptake of a chemical into a medium, as in the movement of nutrients into organism cells. **Desorption** refers to the release of a chemical from a substrate to which it was attached.

5. **Bioaccumulation** refers to the uptake of a chemical by an organism through all routes of exposure, including ingestion, inhalation, and cutaneous absorption. Thus, bioaccumulation is a general term that encompasses two additional concepts, bioconcentration and biomagnification. **Bioconcentration** refers to the uptake of a chemical by an aquatic organism from water alone. **Biomagnification** is the increase in chemical concentration in organism tissues through successively higher trophic levels resulting from chemical transfer in food.

6. Bioaccumulation depends upon bioavailability, i.e., the availability of a chemical in the environment for uptake by organisms. For example, a chemical contaminant that is tightly bound to sediment particles may not be available to organisms exposed to that sediment, regardless of the concentration of contaminant in the sediment. On the other hand, a physical disturbance resulting in sediment resuspension may increase desorption of that contaminant from sediment particles to water, and thus increase the bioavailability of the contaminant to water column organisms. The following sections examine the physical, chemical, environmental, and biological factors that can influence bioaccumulation either directly, or indirectly by increasing or decreasing bioavailability.

Factors Influencing Bioaccumulation

7. Bioaccumulation of chemicals in the aquatic environment can be affected by numerous properties of and processes involving the chemicals themselves, their environment, and the organisms exposed to them. The primary determinants of bioaccumulation are thermodynamic influences, especially

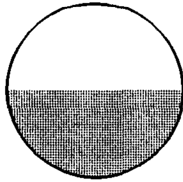
fugacity and equilibrium partitioning; and kinetic influences, i.e., processes affecting rates of chemical uptake and elimination. Thermodynamic influences include primarily chemical and environmental factors, whereas kinetic influences are mainly biological factors.

Thermodynamic influences

8. To better understand chemical mobilities such as bioaccumulation in the environment, one can think of an ecosystem as divisible into various phases or compartments. An aquatic system, for example, can be thought of as having primarily water, sediment, and biota compartments. A chemical contaminant can move among the compartments and will have a certain affinity for each compartment. The lower the affinity of a chemical for a compartment, the greater will be its tendency to escape from that compartment. **Fugacity** (from the Latin *fuga*, "flight") is a measurement of this escaping tendency. Fugacity is measured in units of pressure. Mackay (1979) likened fugacity to temperature: just as heat always diffuses from high to low temperature, so mass (as of a chemical) always diffuses from high to low fugacity. Diffusion continues to occur from one compartment to another until **equilibrium** is established and the fugacity (or temperature, in the case of heat exchange) of both compartments is the same. Thus, at chemical equilibrium, there is no net exchange of chemical mass between the two compartments, and the fugacities of the compartments are equal. This does not imply that the mass (or concentration) of chemical in one compartment is equal to the mass (or concentration) of that chemical in the other compartment. Likewise, at thermal equilibrium, the temperature of the two compartments is the same, but the amount of heat stored in each compartment is not necessarily the same.

9. Fugacity relationships are illustrated in Figure 1 for benzene in a two-phase system consisting of octanol and water. Octanol and water are nearly insoluble in each other and thus may be considered immiscible. If one fills a container partly with octanol and partly with water, shakes the container, and then lets it rest, it will equilibrate as two separate layers with the octanol over the water. A small amount of water will exist in the octanol layer, and a little octanol will be in the water. When equilibrium exists between the octanol and the water (1), the fugacity of water in octanol (f_o^w) will be equal to the fugacity of octanol in water (f_w^o). If an organic compound, benzene, is then introduced into the water (2), f_o^w remains equal to f_w^o, but the fugacity of benzene in octanol (f_o^B) does not equal the fugacity of benzene in water (f_w^B). Because $f_w^B > f_o^B$, benzene "escapes" from the water to

OCTANOL



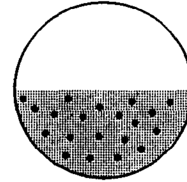
WATER

$f_O^W = f_W^O$

EQUILIBRIUM

a. Equilibrium between octanol and water

OCTANOL



WATER & BENZENE

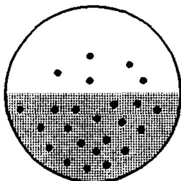
$f_O^W = f_W^O$

$f_O^B \neq f_W^B$

DISEQUILIBRIUM

b. Benzene introduced into the water, resulting in disequilibrium

OCTANOL & BENZENE



WATER & BENZENE

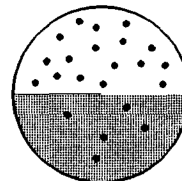
$f_O^W = f_W^O$

$f_O^B \rightarrow f_W^B$

DISEQUILIBRIUM

c. Benzene diffusing from water to octanol

OCTANOL & BENZENE



WATER & BENZENE

$f_O^W = f_W^O$

$f_O^B = f_W^B$

EQUILIBRIUM

d. Equilibrium established for benzene in water and octanol

Figure 1. Fugacity relationships for a two-phase system containing octanol and water

the octanol (3) until equilibrium is reached (4). At equilibrium $f_o^B = f_w^B$, but the concentration of benzene is much greater in octanol than in water because benzene has a greater affinity for (i.e., is more soluble in) octanol than water. In other words, octanol has a greater "containing ability" for benzene than does water.

10. At low concentrations characteristic of chemical contaminants in the environment, fugacity and concentration are linearly related by a proportionality constant that quantifies the ability of a compartment to contain a chemical. This is the fugacity capacity constant Z (Mackay and Paterson 1981):

$$C = Zf \tag{1}$$

where C is the concentration of a chemical in a compartment and f is the fugacity of the chemical in that compartment. In a system consisting of several compartments, a chemical will reach the highest concentration in that compartment for which Z is the highest.

11. The distribution of a chemical between two compartments or phases is referred to as partitioning. The **partition coefficient** is a mathematical constant that describes the concentration differential between the two compartments at equilibrium, i.e., **equilibrium partitioning**. Some examples of partition coefficients that are useful in describing the behavior of chemicals in the environment include:

- K_{ow} Octanol:water
- K_{oc} Soil or sediment organic carbon:water
- S Pure chemical:water (aqueous solubility)
- K_B Organism:water (bioconcentration factor)
- H Air:water (Henry's Law constant)

Figure 2 illustrates the interrelationships between chemical concentration in water and various other compartments, as described by partition coefficients. The compartments include several environmental phases (air, biota, lipid, sediment/soil, suspended particulates, organic carbon), a pure solute (i.e., the chemical of interest), and a pure solvent (octanol). Each compartment has

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* For convenience, equations are listed in numerical order in Appendix B.

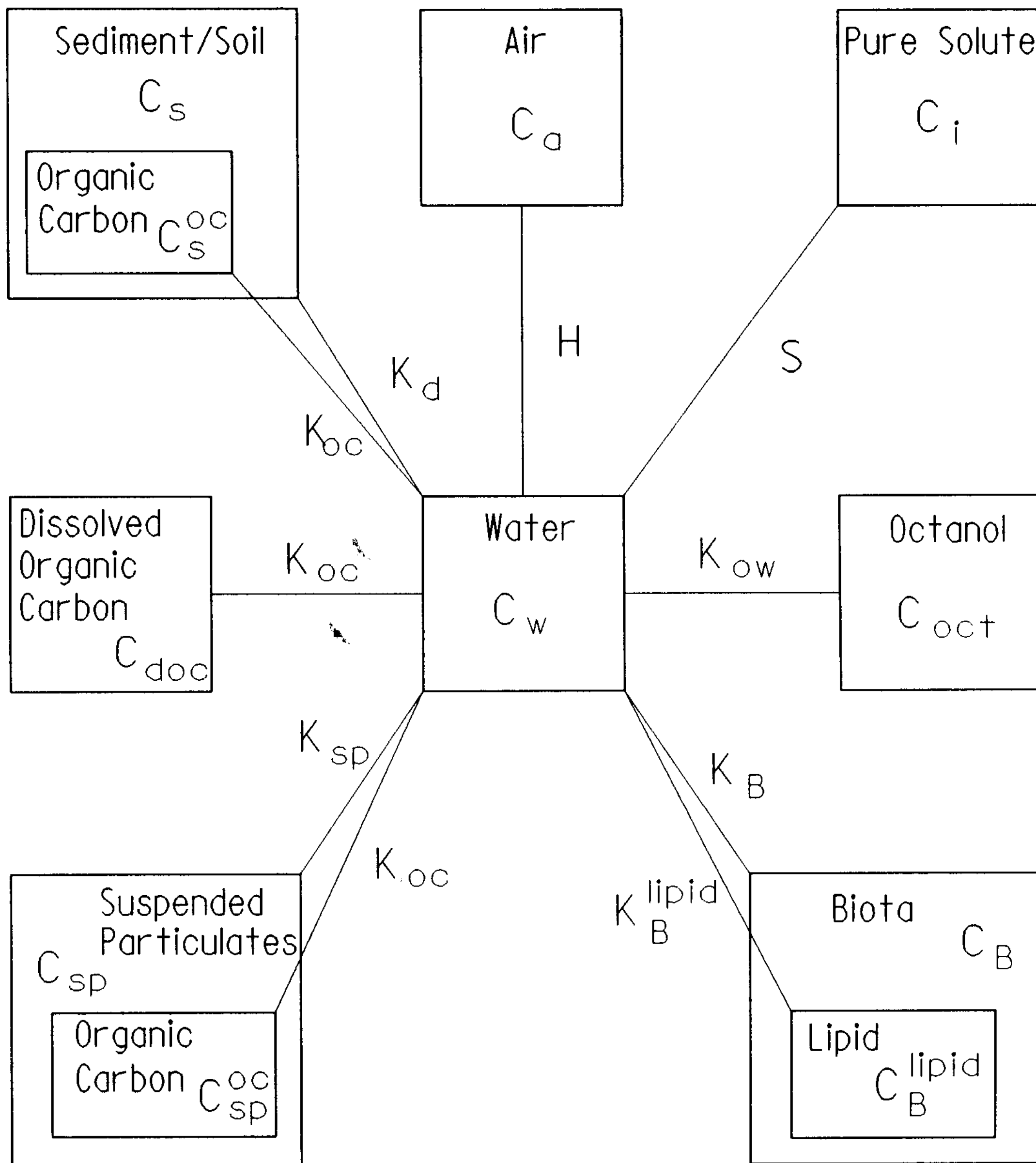


Figure 2. Interrelationships between chemical concentrations in water (C_w) and various other compartments (C_a, C_i, C_{oct}, C_B, C_B^{lipid}, C_{sp}, C_{sp}^{oc}, C_{doc}, C_s, C_s^{oc}). Chemical concentrations in the compartments are related to each other by partition coefficients (H, S, K_{ow}, K_B, K_B^{lipid}, K_{oc}, K_{sp}, K_d)

a concentration of chemical (C_w, C_a, C_i, C_{oct}, C_B, C_B^{lipid}, C_{sp}, C_{sp}^{oc}, C_{doc}, C_s, C_s^{oc}), and is connected to water by partition coefficients (H, S, K_{ow}, K_B, K_B^{lipid}, K_{oc}, K_{sp}, K_d).

12. Fugacity and equilibrium partitioning are **thermodynamic** determinants of bioaccumulation, i.e., they determine how much chemical will be in each compartment when equilibrium is reached, but not the rate at which the transfer takes place. Transfer rates such as rate of uptake and rate of elimination by organisms are **kinetic** processes that will be discussed in the next section.

Kinetic (rate-influencing) processes

13. The primary rate-influencing or kinetic processes for chemical transfer in the aquatic environment include desorption of chemical from sediment, uptake of chemical from water, uptake of chemical from food, metabolism of chemical by an organism, and excretion of chemical by an organism. One may envision a simple chemical transfer process as follows. If sediment is considered to be the main repository for a chemical in the aquatic environment, then the chemical will desorb from sediment to water at some rate. Organisms take up the chemical from water, or perhaps directly from sediment in some cases, and store some of the chemical in their tissues (bioaccumulation). These organisms may then become a source of the chemical to higher trophic level organisms preying on them (biomagnification). An organism will eliminate some of the chemical through respiration and excretion; some of it will be broken down or **biotransformed** by metabolic processes. Figure 3 illustrates a generalized chemical transfer process for lipophilic (fat-soluble) chemicals in the aquatic environment.

14. The rates at which a chemical is taken up and eliminated by an aquatic organism are described by **rate constants**. The basic model for this mathematical relationship is illustrated in Figure 4, where C_w is again the concentration of a chemical in water, C_T is the concentration of the chemical in the tissues of an organism, k_1 is the **uptake rate constant**, and k_2 is the **elimination rate constant**. Rate constants are important in equilibrium partitioning calculations, and will be discussed in more detail in the section titled "Kinetics of Uptake and Elimination" in Part II.

Chemical properties

15. Bioaccumulation of chemical contaminants in the aquatic environment is affected by several properties of the chemicals themselves, including hydrophobicity, solubility, stability, ionizability, and stereochemistry.

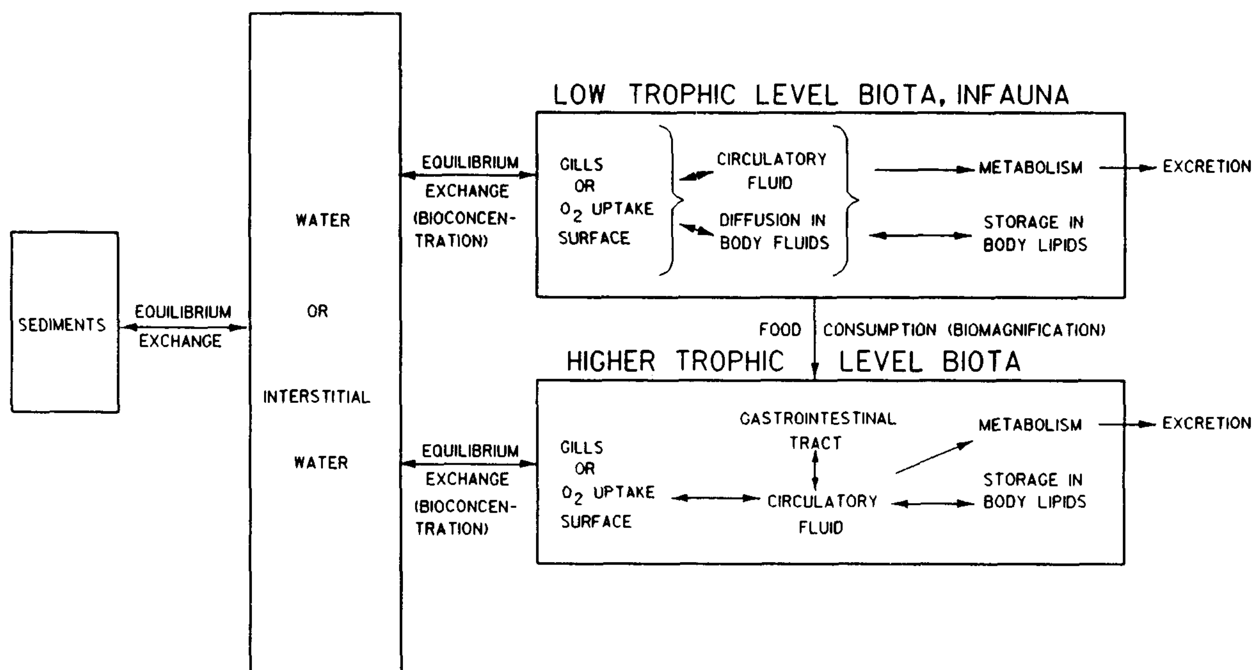


Figure 3. Diagram of routes of uptake and clearance of lipophilic chemicals by aquatic biota (reprinted with permission from Connell 1990)

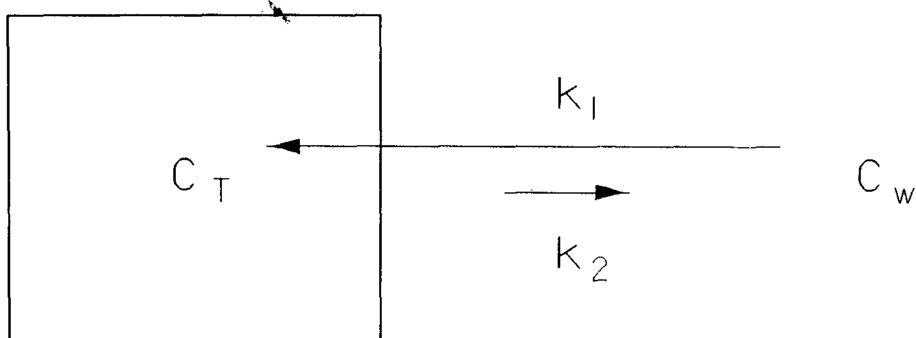


Figure 4. Generalized model for chemical uptake and elimination in an aquatic system, where C_w = chemical concentration in water, C_T = chemical concentration in an organism, k_1 = uptake rate constant, and k_2 = elimination rate constant

Hydrophobicity, which means "fear of water," is a characteristic of uncharged (neutral, nonpolar) organic chemicals. Water molecules are polar and highly charged, and will link up around a neutral molecule in a "shaky cage" structure that requires energy to maintain (Horne 1978). Water will tend to expel the neutral molecule to any available less-energetic phase, such as mineral surfaces (e.g., sediment or suspended particulates), organism lipids, organic solvents, or other associations of neutral molecules (e.g., dissolved organic matter) (Figure 2). Thus, water has low ability to contain hydrophobic

chemicals, and when such chemicals are added to water, their escaping tendency (fugacity) is high.

16. Hydrophobicity generally increases with increasing molecular weight, molecular surface area, and molecular volume of neutral chemicals. The degree of hydrophobicity of a chemical can be described by its octanol:water partition coefficient (K_{ow}). Because K_{ow} for organic chemicals spans many orders of magnitude, it is usually expressed on a $base_{10}$ logarithmic scale. Log K_{ow} (also referred to as log P) of hydrophobic chemicals ranges from about 2 to 10. In other words, at equilibrium, hydrophobic chemicals will concentrate in octanol as opposed to water in ratios ranging from about 100:1 to 10,000,000,000:1, depending largely on the size and lack of charge of the molecule.

17. Octanol does not occur naturally in the aquatic environment, so what is the significance of log K_{ow} in describing the behavior of hydrophobic chemicals in an aquatic system? First, octanol:water partition coefficients have been measured or estimated for thousands of different organic chemicals, and thus provide a broad database for comparisons of chemical behavior in water. Second, organic chemicals are soluble in octanol to about the same extent as they are soluble in organism lipids; thus, octanol is a good surrogate for lipid. Because neutral organic chemicals accumulate in organism lipids, log K_{ow} can provide a good indication of the tendency of a chemical to bioconcentrate and bioaccumulate. However, the relationship between log K_{ow} and the bioconcentration factor (log K_B) is not linear (Figure 5). Log K_B does tend to increase in a linear fashion with log K_{ow} over the range of log K_{ow} = 2 to 6, after which log K_B begins to decrease with increasing hydrophobicity. This means that bioaccumulation calculations based on linear relationships with log K_{ow}, as presented in Part III of this paper, will likely be increasingly inaccurate for neutral organic chemicals whose log K_{ow}s are increasingly greater than 6. Included in this category are some highly hydrophobic environmental contaminants, such as the polychlorinated biphenyl (PCB) congeners having seven or more chlorine atoms and log $K_{ow} > 7$.

18. **Solubility** is defined as the mass of substance contained in a solution that is in equilibrium with an excess of the substance (CRC Press, Inc. 1982). Solubility in water is measured by the pure chemical:water partition coefficient S. Aqueous solubility is inversely related to hydrophobicity. Thus, highly bioaccumulating chemicals will be those with extremely low water solubilities. In actuality, high water solubility favors rapid uptake of

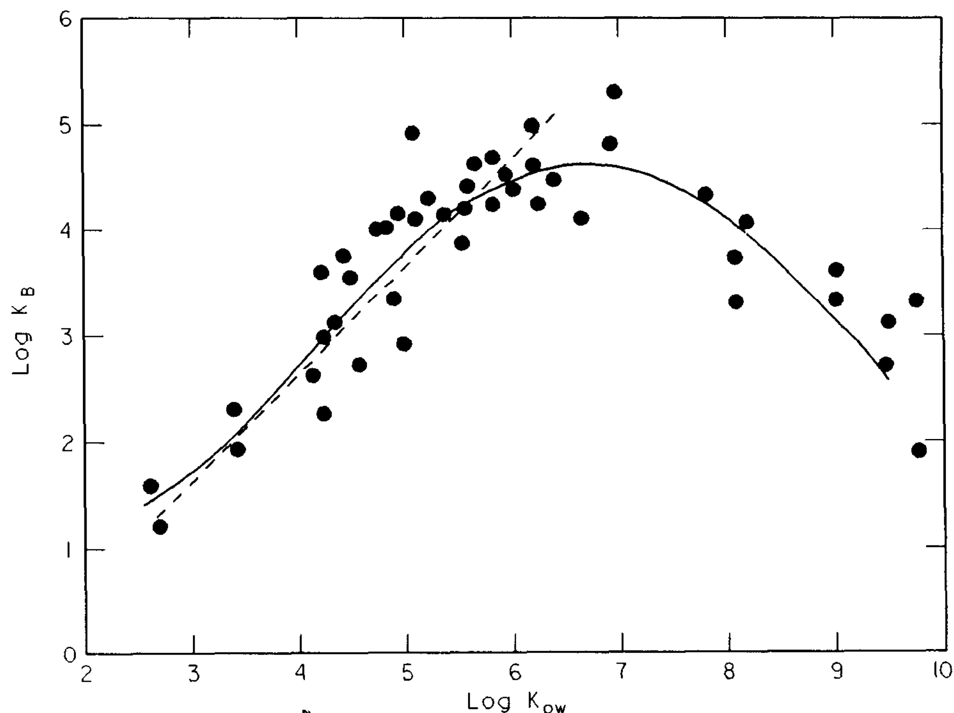
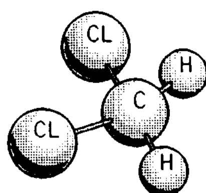


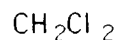
Figure 5. Relationship between log K_B and log K_{ow} for bioconcentration (reprinted with permission from Connell 1990)

chemicals by organisms but at the same time favors rapid elimination. Such chemicals do not have a chance to accumulate in organism tissues. Neutral organic compounds are increasingly insoluble in water as their molecular mass increases; these compounds tend to be the most highly bioaccumulating. Figure 6 illustrates the relationship between molecular weight, hydrophobicity, aqueous solubility, and the bioconcentration factor for some example organic contaminants. Heavy metals such as cadmium, mercury, and lead may occur in ionized forms that are soluble in water, but these substances bind with organism tissues and thus are actively bioaccumulated.

19. **Stability** of a chemical refers to its resistance to degradation, and is an important prerequisite for bioaccumulation. Chemicals that are easily broken down and eliminated by organisms do not bioaccumulate; examples include the organophosphate insecticides such as parathion and malathion, and the polynuclear aromatic hydrocarbons (PAHs) in fishes. However, some invertebrates such as bivalve mollusks and certain amphipods have low metabolizing capability for PAHs and do bioaccumulate them. The presence of electron-withdrawing substituents on organic molecules tends to stabilize them. Chlorines, for example, are bulky, highly electronegative atoms that tend to



METHYLENE CHLORIDE
(DICHLOROMETHANE)



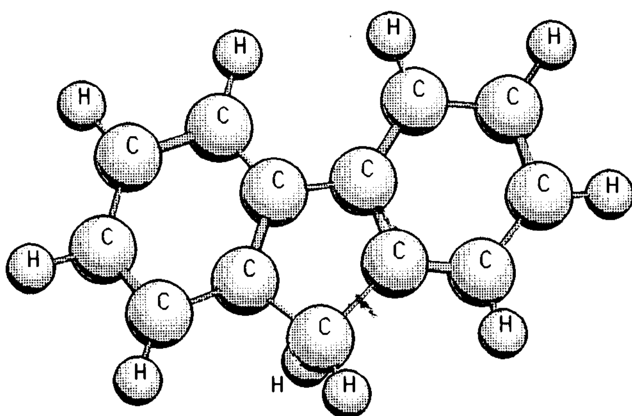
mw = 84.93

log K_{ow} = 1.3

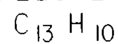
S = 20,000 mg/L @20°C

K_B = does not bioaccumulate

Source = common solvent



FLUORENE



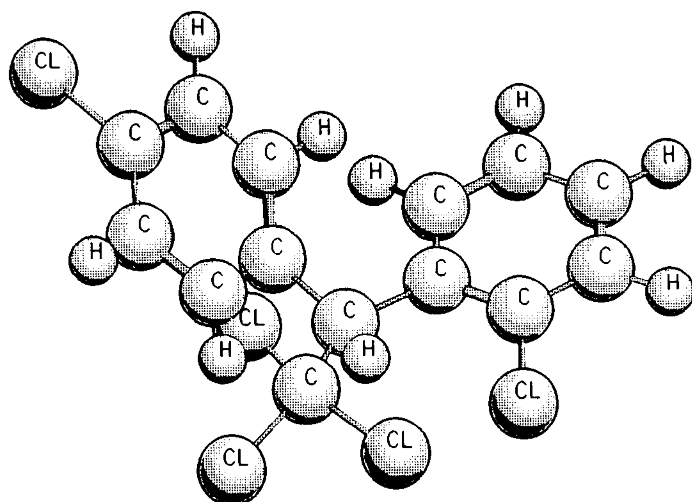
mw = 166.2

log K_{ow} = 4.38

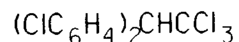
S = 1.9 mg/L @25°C

K_B = 1,300

Source = coaltar, combustion product



DICHLORO-DIPHENYL-
TRICHLOROETHANE
(*o,p'*-DDT)



mw = 354.5

log K_{ow} = 5.75

S = 0.003 mg/L @25°C

K_B = 37,000

Source = contact insecticide

Figure 6. Examples of neutral organic chemicals showing the relationship among size (molecular weight, mw), hydrophobicity (log K_{ow}), solubility(s), and bioconcentration factor (K_B)

protect the nucleus of an organic molecule from chemical attack. Highly chlorinated organic compounds such as some of the PCBs bioaccumulate to high levels because they are easily taken up by organisms and cannot be readily broken down and eliminated. Other stable organic compounds that are frequently contaminants in the aquatic environment include organochlorine pesticides (e.g., dichloro-diphenyl-trichloroethane (DDT), dioxins, and dibenzofurans.

20. The presence of functional groups such as carboxylic acid, hydroxyl, phenolic, or ether or ester linkages in or on a molecule tends to make the molecule chemically reactive, thereby diminishing its stability in the environment.

21. Metals are inherently stable because they are elemental in nature. Nevertheless, the forms in which a trace element can occur vary greatly in their bioavailability. Metals within the crystal lattice of minerals are very stable but clearly are not bioavailable. On the other hand, metals dissolved in surface and interstitial waters can remain readily bioavailable to organisms at many trophic levels (Patrick, Gambrell, and Khalid 1977). Metals are taken up by organisms either as ions in solution or as organometallic complexes. Complexation of metals may facilitate bioaccumulation by increasing bioavailability. For example, the organometalloid methyl mercury is more bioavailable (and more toxic) to organisms than inorganic forms of mercury. Organometalloids that are taken up by organisms can hydrolyze, allowing the free metal ion to bond with reactive biochemical molecules in organism tissues.

22. **Ionizability** refers to the ability of a chemical to form ions (electrically charged particles) in solution. The ions may be positively charged (cations) or negatively charged (anions). The process of splitting into ions is called **dissociation**. Neutral organic chemicals do not ionize. Metals that are bioavailable are generally those that are present as cations in solution. If cadmium ions (Cd^{++}), for example, are present in the water that an aquatic organism respires, they can bind with biological materials and will tend to bioaccumulate.

23. Weak organic acids and bases (Table 1) are present in ionized or nonionized forms in natural waters to an extent determined by their acid dissociation constants, *pKa*. The *pKa* is the pH* at which a weak acid or base is

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* For additional discussion of pH in relation to bioaccumulation, refer to the next section (Environmental Factors).

50 percent dissociated, i.e., [nonionized] = [ionized]. The degree of dissociation of an acid or base is determined by the pH of the solution containing the acid or base. The nonionized form of a weak acid or base is the bioavailable form.

24. The relative amounts of ionized and nonionized forms of a weak acid or base in solution can be calculated using derivations of the Henderson-Hasselbach equation:

$$\text{For acids: } pKa - \text{pH} = \log([\text{nonionized}]/[\text{ionized}]) \quad (2)$$

$$\text{For bases: } pKa - \text{pH} = \log([\text{ionized}]/[\text{nonionized}]) \quad (3)$$

where the brackets indicate concentrations. To demonstrate these calculations, take chloroacetic acid in seawater as an example. The pH of seawater is about the same as that of blood plasma, 7.4. Chloroacetic acid has a low *pKa*, 2.85 (Table 1). Using Equation 2,

$$2.85 - 7.4 = \log([\text{nonionized}]/[\text{ionized}])$$
$$-4.55 = \log([\text{nonionized}]/[\text{ionized}])$$
$$[\text{nonionized}]/[\text{ionized}] = 2.82 \times 10^{-5} = 1{:}282{,}000$$

Therefore, chloroacetic acid is highly ionized in seawater. Because the nonionized form is the bioavailable form, chloroacetic acid in seawater would not be bioaccumulated by marine organisms (at least not through the gill surfaces). However, if chloroacetic acid were ingested by an organism having a stomach pH of 2, then

$$2.85 - 2 = \log([\text{nonionized}]/[\text{ionized}])$$
$$0.85 = \log([\text{nonionized/ionized}])$$
$$[\text{nonionized}]/[\text{ionized}] = 7.08 = 7.08{:}1$$

giving a very slight preference to the nonionized form, and thus some bioaccumulation through ingestion. As a second example, one can perform the calculations for a weak acid having a high *pKa*. Using *o*-cresol (*pKa* = 10.2) in seawater (pH = 7.4) with Equation 2:

Table 1

Dissociation Constants (*pKa*) of Organic Acids and Bases in Aqueous Solution

Acid	*pKa*	Acid	*pKa*
Adipamic	4.63	*o*-Nitrophenol	7.17
Adipic (Step 1)	4.43	*m*-Nitrophenol	8.28
Adipic (Step 2)	5.41	*p*-Nitrophenol	7.15
Benzoic	4.19	*o*-Phthalic (Step 1)	2.89
Cacodylic	6.19	*o*-Phthalic (Step 2)	5.51
Chloroacetic	2.85	*m*-Phthalic (Step 1)	3.54
o-Chlorobenzoic	2.92	*m*-Phthalic (Step 2)	4.60
m-Chlorobenzoic	3.82	*p*-Phthalic (Step 1)	3.51
p-Chlorobenzoic	3.98	*p*-Phthalic (Step 2)	4.82
o-Chlorophenoxyacetic	3.05	Resorcinol	9.81
m-Chlorophenoxyacetic	3.10	Trichloroacetic	0.70
o-Chlorophenylacetic	4.07	Trichlorophenol	6.00
m-Chlorophenylacetic	4.14	Trihydroxybenzoic (2,4,6-)	1.68
o-Cresol	10.2		
m-Cresol	10.01	**Base**	***pKa***
p-Cresol	10.17		
Dichlorophenol (2,3-)	7.44	Aniline	4.63
Dinitrophenol (2,4-)	3.96	Aniline, *o*-chloro	2.65
Dinitrophenol(3,6-)	5.15	Aniline, *m*-chloro	3.46
o-Monochlorophenol	8.49	Aniline, *p*-chloro	4.15
m-Monochlorophenol	8.85	Aniline, 3-chloro-N,N-dimethyl	3.837
p-Monochlorophenol	9.18	Aniline, 2,4-dichloro	2.05
Nitrobenzene	3.98	Benzidine	4.66
o-Nitrobenzoic	2.16	Biphenyl, 2-amino	3.82
m-Nitrobenzoic	3.47	Naphthalene, dimethylamino	4.566
p-Nitrobenzoic	3.41		

$$10.2 - 7.4 = \log([\text{nonionized}]/[\text{ionized}])$$

$$2.80 = \log([\text{nonionized}]/[\text{ionized}])$$

$$[\text{nonionized}]/[\text{ionized}] = 631:1$$

This ratio favors the nonionized form, meaning that *o*-cresol would be bioaccumulated from seawater. If *o*-cresol were ingested (stomach pH = 2), then

$$10.2 - 2 = \log([\text{nonionized}]/[\text{ionized}])$$

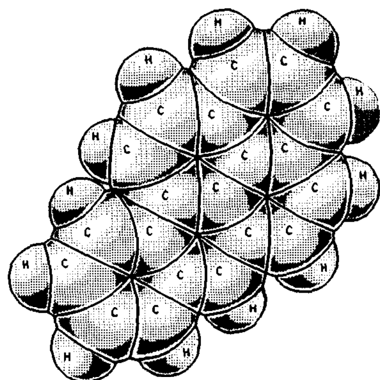
$$8.2 = \log([\text{nonionized}]/[\text{ionized}])$$

$$[\text{nonionized}]/[\text{ionized}] = 158{,}489{,}319:1$$

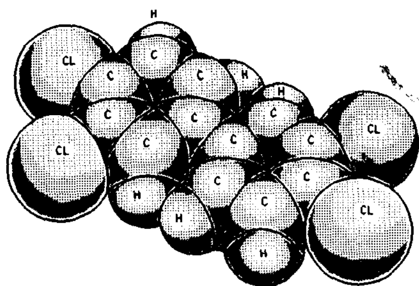
In this case, the nonionized form is very highly favored, and *o*-cresol would be strongly absorbed through the stomach.

25. **Stereochemistry** refers to the spatial configuration (three-dimensional shape) of a molecule, and affects its tendency to bioaccumulate.

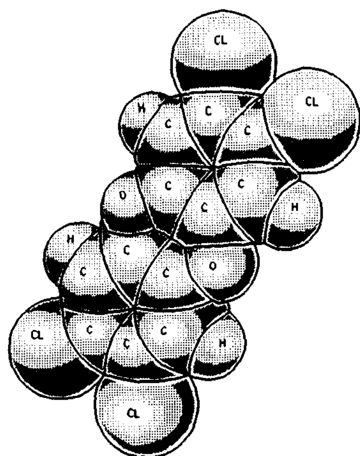
PLANAR MOLECULES



Benzo[a]pyrene
mw = 252.3

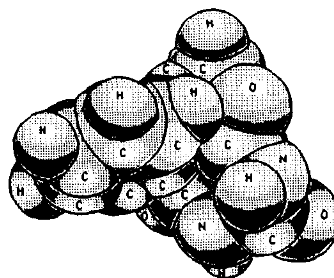


3,3',4,4' - Tetrachlorobiphenyl
mw = 292.0

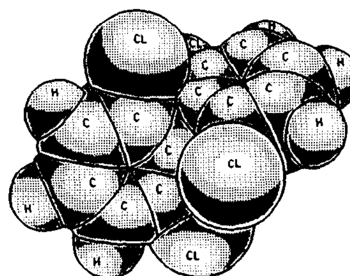


2,3,7,8 - TCDD
mw = 321.97

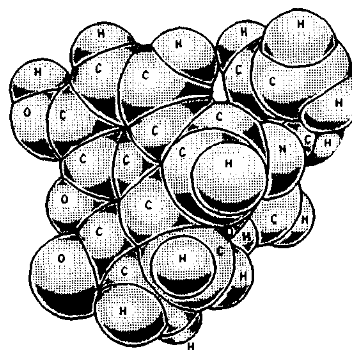
GLOBULAR MOLECULES



Phenobarbital
mw = 232.2



2,2',6,6' - Tetrachlorobiphenyl
mw = 292.0



Naloxone
mw = 327.37

Figure 7. Diagrams of planar molecules versus globular molecules of similar molecular weight

Planar (flat) molecules tend to bioaccumulate more highly than globular molecules of the same molecular weight. Planar molecules such as PAHs, dioxins, and certain PCB congeners lacking *ortho*-chlorine substitution (Figure 7) tend to be more lipid soluble than globular molecules of similar molecular weight. Planarity in some cases may enhance toxicity as well as bioaccumulation (McFarland and Clarke 1989).

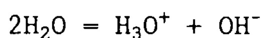
26. Large neutral molecules may not bioaccumulate because transport across biological membranes is restricted by the size of the molecule. This hypothesis has been referred to as steric hindrance (Opperhuizen et al. 1985). Neutral molecules that have cross-sectional dimensions greater than about 9.5 Å (.00095μm) are thought to be sterically hindered in their ability to penetrate the polar surfaces of cell membranes in fish gut or gill tissues. Examples of such molecules include octachlorodibenzo-*p*-dioxin (9.8 Å, .00098 μm) and decabromobiphenyl (9.6 Å, .00096 μm), which essentially do not bioaccumulate.

Environmental factors

27. A number of factors relating to sediment and water influence bioaccumulation of chemical contaminants in the aquatic environment. Factors that will be discussed in this paper include acidity/basicity and redox potential, sediment organic carbon, kinetics of adsorption and desorption, oil and grease, sediment particle size, sediment suspension, particle interaction effect, dissolved organic carbon, hardness, and salinity. Most of these environmental factors affect the concentrations of chemicals in various compartments more so than the rates of chemical transfer among compartments; i.e., they are thermodynamic rather than kinetic influences on bioaccumulation. Some factors have a more pronounced effect on the bioavailability of metals; other factors, on the bioavailability of neutral organic contaminants.

28. The **acidity** or **basicity (pH)** and the **oxidation-reduction (redox) potential (Eh)** of an aqueous medium or sediment can be of considerable importance in controlling chemical reactions in the aquatic environment. Eh and pH have a strong influence on the bioavailability and bioaccumulation of metals, weak organic acids, and weak organic bases, but little effect on neutral organic chemicals.

29. Water dissociates to a very limited extent into protons (H^+), normally present as hydronium ions (H_3O^+), and hydroxyl ions (OH^-) as described by the chemical reaction:



$$2H_2O = H_3O^+ + OH^-$$

pH is defined as the negative logarithm ($base_{10}$) of the hydronium ion concentration:

$$pH = -\log [H^+] = \log 1/[H^+]$$

and ranges from 0 to 14, with pH 7 being neutral, < 7 acidic, and > 7 basic (alkaline). The strongest acids and bases are those with pH approaching 0 and 14, respectively.

30. Redox potential (Eh) refers to the tendency of a solution to be oxidized or reduced. Oxidation is defined as any process that increases the proportion of oxygen or acid-forming elements or radicals in a compound. Reduction is any process that increases the proportion of base-forming elements or radicals in a compound. Reduction is also the gaining of electrons by an atom, an ion, or an element, thereby reducing the positive valence of that which gained the electrons (CRC Handbook of Chemistry and Physics 1982).

31. The oxidation potential of a reaction is the drop in potential, measured in millivolts (mv), occurring when a neutral atom is oxidized (ionized) to a cation, or an anion to a neutral atom, or an ion to a more highly charged state (e.g., chromous ion, Cr^{+2}, to chromic ion, Cr^{+3}). The reduction potential of a reaction is the reverse, i.e., the increase in potential, in mv, involved in a reduction reaction (e.g., Cr^{+3} to Cr^{+2}). A standard redox potential is developed by a solution containing molar concentrations of both forms of the element, ions, or radicals involved in the reaction. Standard redox potentials for some reactions of chromium (Cr) are:

	Reaction	Eh, mv
a.	$Cr^{+2} + 2e^- \longleftrightarrow Cr$	- 557
b.	$Cr^{+3} + e^- \longleftrightarrow Cr^{+2}$	- 410
c.	$Cr^{+3} + 3e^- \longleftrightarrow Cr$	- 740

These are reduction reactions in which the chromous ion (Cr^{+2}) gains two electrons and is reduced to metallic chromium (a.), the chromic ion (Cr^{+3}) gains one electron and is reduced to chromous (b.), or gains three electrons and is reduced to metallic chromium (c.).

32. Natural waters are weakly to strongly oxidized (Eh ranging from about 300 to 600 mv) and mildly acidic to mildly alkaline (pH ranging from about 5.0 to 8.5). Sediments are generally reduced (Eh ranging from about 100 to -400 mv) and nearly neutral in pH (pH ranging from about 6.5 to 7.5) (Patrick, Gambrell, and Khalid 1977). The combination of oxidizing conditions

and low pH in natural waters (and at the sediment surface) favors the presence of metals as ions in solution, and tends to increase metal bioavailability and bioaccumulation. These circumstances also favor precipitation of hydrous oxides of manganese and iron, along with possible coprecipitation of solubilized free metal ions, thus taking them out of solution. However, the two processes (solubilization and precipitation) are in opposition. Whether bioaccumulation is favored or not depends largely on the relative concentrations of the hydrous metal oxides and heavy metal ions.

33. As a general rule, free metal ions tend to be present in greater abundance and thus are more bioavailable at low pH and under oxidizing conditions. Under reducing conditions, metals are present largely as insoluble sulfides and are generally less bioavailable. Trace metals that are associated with sediments but are not bound in the sediment crystal matrix are present as ions, complexes, or precipitates. Aqueous concentration of the free ions is regulated by solubility of the precipitates under prevailing conditions of Eh and pH.

34. **Sediment organic carbon** (as measured by total organic carbon or **TOC**) is the primary storage compartment for neutral organic chemicals in sediments. TOC consists mainly of humic (decayed plant) materials, with which neutral organic chemicals tend to associate. TOC generally constitutes about 1 to 4 percent of silty harbor sediments and can be as much as 10 to 20 percent of navigation channel sediments. Very sandy sediments may contain less than 1 percent TOC.

35. Organic carbon behaves as though it were an organic solvent in competition with the lipids of organisms for containment of any neutral organic chemicals that are present (Lambert, Porter, and Schieferstein 1965). Thus, TOC is a major determinant of the bioaccumulation potential of neutral organic chemicals in sediment. For a given concentration of chemical in sediment, low TOC favors increased bioaccumulation potential and high TOC favors the reverse. Metals are also associated with TOC, primarily by active bonding with functional groups rather than by passive equilibrium. Metal bioavailability is affected by complex interactions involving additional factors such as Eh and pH.

36. Because neutral organic chemicals are present primarily in the organic carbon fraction of sediments, one can **normalize** their concentration on sediment TOC, i.e., divide the concentration of the chemical in the whole sediment by the decimal fraction TOC content (Karickhoff, Brown, and Scott

1979). Such normalization can give a better idea of the amount of chemical in a sediment that is actually available to organisms, and provides the basis for determining bioaccumulation potential, as described later in this paper. The effect of TOC normalization on neutral organic chemical concentration in sediments of different TOC contents is exemplified in Table 2.

Table 2

TOC Normalization of Neutral Organic Chemical Concentrations in Sediments of Different TOC Content

Contaminant Concentration in Sediment	TOC-Normalized Contaminant Concentration			
	1% TOC	5% TOC	10% TOC	20% TOC
0.001 ppm	0.1 ppm	0.02 ppm	0.01 ppm	0.005 ppm
0.01 ppm	1.0 ppm	0.2 ppm	0.1 ppm	0.05 ppm
0.1 ppm	10 ppm	2.0 ppm	1.0 ppm	0.5 ppm
1.0 ppm	100 ppm	20 ppm	10 ppm	5.0 ppm
10 ppm	1000 ppm	200 ppm	100 ppm	50 ppm
100 ppm	10000 ppm	2000 ppm	1000 ppm	500 ppm

37. When TOC content is very low, the TOC-normalized contaminant concentrations are greatly increased relative to their sediment concentrations. For example, 100 parts of contaminant per million parts of sediment normalizes to 10,000 parts of contaminant per million parts of TOC when TOC content is only 1 percent. The same 100 parts of contaminant per million parts of sediment normalizes to just 500 parts of contaminant per million parts of TOC when TOC content is 20 percent.

38. **Kinetics of adsorption and desorption** are important in understanding environmental influences on bioavailability, particularly of neutral organic chemicals. Sediment particle size, TOC, and relative hydrophobicity of individual chemicals are major factors influencing rates of sorption. For metals, rates of sorption processes are strongly Eh and pH dependent. Adsorption and desorption of hydrophobic contaminants to and from sediments have a rapid component and a slow or resistant component (Karickhoff and Morris 1985). The rapid component occurs in a matter of minutes and accounts for about 10 to 60 percent of the sorption capacity of sediment particles. The resistant component of adsorption and desorption takes place over a period of days to weeks in laboratory experiments. Highly hydrophobic chemicals may require more than a year to completely desorb from a sediment. Kinetics of

desorption, then, are of particular interest in estimating the bioavailability of hydrophobic chemicals from sediments. Estimation methods that rely on equilibrium distribution of chemicals among environmental compartments (such as the methods described in Part III of this paper) may overestimate the bioavailable fraction of a chemical in sediments, depending on the amount of time allowed for equilibration.

39. **Oil and grease** is a nonspecific determination often included in sediment chemical inventories, and is composed primarily of non-bioaccumulating alkanes. However, in sediments, oil and grease may affect the bioavailability of other chemicals that do bioaccumulate. When present in sufficiently high concentrations to constitute a discrete phase (such as oil droplets or tar balls), oil and grease may concentrate organic chemicals in a manner similar to sediment organic carbon. In fact, oil and grease has recently been estimated to be about 10 times more effective as a sorptive phase for hydrophobic chemicals than is TOC (Boyd and Sun 1990). In effect, oil and grease could add incrementally to the TOC pool in a sediment, thus reducing the bioavailability of organic chemical contaminants to organisms. However, the mass contributed by total oil and grease in sediments is usually insignificant compared with the mass represented by humic TOC, and can usually be disregarded.

40. **Sediment particle size** influences the sorption of both metals and neutral organic chemicals. As sediment particle size decreases, the surface area of the particles per unit mass of sediment increases. Increasing surface area increases the number of negatively charged sites with which cations may bond, thus increasing the adsorption of metals. Increasing surface area also increases the number of sites for adsorption of neutral organic chemicals by means of van der Waals/London forces. Sediment particulates have coatings of humic matter, and most of the sediment organic carbon is associated with the finer particles. For these reasons, chemical contaminants in sediments are associated primarily with the fine-grained fraction of sediments, and the coarser-grained sediments (sands) tend to be fairly clean in terms of chemical contamination. Benthic organisms that dwell in or ingest fine-grained material are potentially exposed to higher environmental concentrations of chemicals than are those in coarse-grained sediments, and usually reflect this in their higher bioaccumulation (Frank, Landrum, and Eadie 1986). The same is true of filter-feeding organisms that select small-sized particulates for ingestion.

41. Dredging and disposal operations that cause **sediment suspension** can, at least transiently, increase the concentrations of associated chemical contaminants in the water column. This increase is not a simple linear function of the mass of sediment suspended because the contaminant-bearing TOC of the fine-grained suspended sediment fraction is typically higher than the TOC of the coarser grained deposited sediment (see discussion of sediment particle size). Nevertheless, when the fugacity of chemicals is less on suspended particulates than it is in the water, the TOC of suspended sediments can act as a scavenger of metals and organic chemicals from solution. This reduces the bioavailability of the chemicals, and in some cases actually reduces the mass of dissolved contaminants in the water (Reilly and Bellis 1983).

42. The water column of an aquatic environment is not simply pure water, but also includes living organisms and non-living substances. The non-living substances are present in a continuum of sizes ranging from suspended particulates to colloids (filterable particles) to dissolved matter (molecular particles). In salt water, the presence of divalent cations of magnesium and calcium (Mg^{++} and Ca^{++}) can cause suspended sediments, colloids, and dissolved organic matter to flocculate and settle from the water column. Under these conditions, low molecular weight organic acids can be precipitated as metal complexes, and trace elements may coprecipitate with flocculated material (Lindberg and Harriss 1977; Luther et al. 1986). These processes tend to reduce bioavailability.

43. Suspension of uncontaminated sediments reduces the bioavailability of dissolved water column contaminants by adsorbing them from solution. On the other hand, suspension of contaminated sediments in clean water increases the exposure of filter-feeding and water-column-dwelling organisms to contaminants. In such cases fugacity favors desorption from particulates to the water, and chemicals including PCBs, kepone, lead, and mercury that are bound with the particulates may be made bioavailable (Brown 1981; Eaton et al. 1983; Rice and White 1987).

44. The desorption of contaminants from suspended sediments apparently increases as the concentration of particles in suspension increases. This **particle interaction effect** may be due to increased collision of suspended particles, releasing chemicals from sites where they are "loosely" sorbed (Mackay and Powers 1987). The effect has resulted in observed inverse correlations between suspended particulate concentrations in water and the particulate:water partition coefficients for both metals and organic chemicals

(O'Connor and Connolly 1980). In bedded sediments where the particles are at rest, partition coefficients are constant, and K_{oc} adequately describes the equilibrium distribution of hydrophobic chemicals with the interstitial water (Di Toro et al., in review). However, in dilute sediment suspensions where particulates are highly organic, K_{ow} rather than K_{oc} best describes hydrophobic chemical partitioning with water. MacKay and Powers (1987) suggested that only about 40 percent of the surface organic carbon of particles makes up the lining of the pores in bedded sediments, and thus only 40 percent of the organic carbon by mass is available for exchange with the interstitial water. This would account for the fact that K_{oc} is only about 40 percent of K_{ow} for most neutral organic chemicals.

45. The particle interaction effect is still not fully explained or accepted in the scientific community. However, the existence of such an effect could have substantial implications for contaminant bioavailability during dredging operations that produce high turbidity. The suspension of high levels of contaminated sediments during disposal operations could conceivably increase the concentration of desorbed chemicals in the water column. Such an effect would amount to an increase in bioavailability for exposed organisms because the amount of unbound chemical present would be greater than expected from simple desorption.

46. **Dissolved organic carbon (DOC)** in the aquatic environment is composed primarily of humic substances produced by the degradation of dead plant material. Humic and fulvic acids make up 40 to 80 percent of DOC. These organic acids are structurally complex colloidal and subcolloidal compounds containing large numbers of functional groups (e.g., phenolic, hydroxylic, and carboxylic acid) and straight and branched alkyl side-chains. The functional groups confer water solubility and also provide binding sites for metal ions in solution. The alkyl chains provide sites for adsorption of hydrophobic chemicals.

47. The concentration of DOC affects bioavailability and bioaccumulation of chemicals by aquatic organisms in a manner similar to that of suspended sediment (Carlberg et al. 1986; Caron, Suffet, and Belton 1985; McCarthy 1983). Bioaccumulation is reduced when metals or neutral organic chemicals are added to water containing uncontaminated DOC. High DOC concentrations in the water column appear to reduce bioaccumulation by adsorbing neutral organic contaminants and making them less available to organisms. The bioavailability of metals such as copper and zinc may increase or decrease

depending on salinity and suspended sediment concentrations. As with contaminated suspended sediment, contaminated DOC can increase organism exposure to chemicals.

48. Water **hardness** refers to the concentration of dissolved salts of calcium and magnesium; in particular, water containing > 85.5 ppm calcium carbonate is considered hard. Increasing hardness decreases the bioavailability, and presumably bioaccumulation, of the toxic forms of metals (Winner and Gauss 1986). The influence of hardness on bioaccumulation of most organic compounds is negligible (Bradley and Sprague 1985).

49. **Salinity** has complex effects, both direct and indirect, on bioaccumulation and bioavailability. Salinity influences physicochemical processes including desorption and solubility, as well as physiological processes such as osmoregulation, membrane permeability, and respiration rate and volume. Factors other than salinity are usually more important in affecting bioaccumulation of contaminants. If a salinity effect on bioaccumulation is present, it will usually be observed at < 1 part per thousand salinity; increasing the salinity does not increase the effect.

50. Increasing salinity tends to decrease the water solubility of neutral organic chemicals, and also decreases the concentration of both particulate and dissolved organic carbon. Recall that bioavailability of neutral organic chemicals is inversely related to TOC and DOC, and thus the decrease in organic carbon with increasing salinity may under some conditions actually enhance bioavailability of neutral organics to organisms.

51. The relationship of salinity to bioaccumulation of metals is more complex and element-specific. Metals in solution have been reported to bioaccumulate to higher concentrations as salinity decreases, but the opposite may also be true. In general, selenium solubility and bioavailability are inversely related to salinity; zinc uptake is unrelated to salinity; copper uptake is erratic and is especially affected by complexation with organic compounds; lead uptake increases with increasing salinity; and cadmium uptake is inversely related to salinity (Burton 1978; Duursma et al. 1986; Frenet 1981; Gambrell, Khalid, and Patrick 1980; Hashimoto et al. 1984; Kuwabara et al., in press; Tedengren, Arner, and Kautsky 1988; Wildish et al. 1980; Wright and Zamuda 1987). Mercury binds very tightly to particles and does not respond to salinity changes, although Clarke, Lutz, and McFarland (1988) noted a slight but statistically significant increase in mercury uptake with increasing salinity by clams exposed to mercury-contaminate suspended

sediment. However, fish exposed to the same sediment under the same conditions did not bioaccumulate mercury.

Biological factors

52. Biological factors in general do not affect the bioavailability of aquatic contaminants, but are of primary importance in influencing the amount of bioaccumulation that takes place, the rate at which it takes place, and to some extent, the source (sediment, water, or food) from which contaminants are taken up by organisms. The following biological factors will be considered in this paper: diet and feeding type, depuration, metabolic rate, biotransformation, mixed-function oxidases, metallothioneins, and lipid content.

53. Uptake of contaminants through **diet** and the role of biomagnification in bioaccumulation were previously thought to be of minimal significance in the aquatic environment (Crump-Wiesner, Feltz, and Yates 1974; Pavlou and Dexter 1979; Kay 1985). Most bioaccumulation was considered to occur from exposure to contaminated sediments and suspended particulates, or directly through bioconcentration from water. However, recent literature assigns a greater role to contaminated food as a major pathway for bioaccumulation of contaminants in aquatic organisms, particularly in pelagic fishes (Thomann and Connolly 1984; Connolly and Pedersen 1988; Oliver and Niimi 1988; van der Oost, Heida, and Opperhuizen 1988; Clarke, Whitman, and Dorkin, in review). Dietary bioaccumulation is favored when the food that an organism consumes is highly contaminated relative to the water that it respires. Connell (1990) noted that biomagnification is likely to be of more significance than bioconcentration in air-breathing aquatic organisms and in long-lived species, especially top predators. Biomagnification is also most likely to occur with persistent chemicals having log $K_{ow} > 5$.

54. Thomann (1989) modeled bioaccumulation of organic chemicals in a simple generic aquatic food chain. The model estimated that food chain biomagnification was not significant for chemicals having log K_{ow} up to ~5 due to decreased uptake and increased excretion. Biomagnification was most significant for chemicals in the log K_{ow} range of 5 to 7, accounting for virtually the entire body burden in top predators at log $K_{ow} = 6.5$, and continued to be of importance for chemicals of log K_{ow} to ~8.

55. Deposit-feeding benthic infaunal invertebrates and bottom-feeding fishes that process sediment or ingest detritus accumulate contaminants primarily through their feeding activities on sediment and organic matter. Thus, **feeding type** and **trophic level** (position in the food chain) are important

determinants of contaminant sources for bioaccumulation. Filter-feeding animals can bioaccumulate contaminants directly from water and from suspended sediment and ingested small organisms. Carnivores, especially top predators, may obtain their contaminant burdens almost entirely through their food (Thomann and Connolly 1984). The relative importance of the various environmental compartments as contaminant sources for bioaccumulation can also change over the life cycle of an organism when the organism occupies different trophic levels or habitats during different stages of development. Many invertebrates, for example, have a planktonic larval stage followed by benthic juvenile and adult stages. Predatory fishes such as lake trout consume invertebrates until the predators reach a certain size, after which they consume fish.

56. Dietary bioaccumulation is dependent on feeding and clearance rates and on the ability of organisms to assimilate chemicals. The assimilation efficiency of predatory fishes for organic contaminants and organometalloids from food ranges from about 65 to 95 percent. Deposit feeders assimilate these contaminants with about 20 to 40 percent efficiency. Filter feeders are intermediate or similar to deposit feeders in their assimilation efficiencies.

57. As contaminant body burdens are increasing through feeding or other modes of uptake, they are also decreasing through elimination. **Depuration** refers to the loss of toxic substances from an aquatic organism by all processes of elimination. The principal processes of elimination are metabolism, excretion, and respiration. Cutaneous elimination also occurs. Some aquatic organisms achieve substantial contaminant depuration during spawning (Guiney et al. 1979), particularly of neutral organic chemicals such as PCBs that accumulate in the gametes (Black, Phelps, and Lapan 1988).

58. When the net loss of a chemical by depuration is equal to the net gain of that chemical by uptake, bioaccumulation is considered to be at steady state. If uptake increases, then bioaccumulation will increase until a new, higher steady state is achieved. Likewise, if an organism moves to conditions of lower contaminant exposure, then depuration will be favored over uptake until a lower steady state is reached. In most cases, depuration is a two-phase process. First, chemicals in the bloodstream or in tissues with high blood exchange (e.g., gills) are eliminated fairly quickly, and then the same chemicals in storage tissues such as fat are mobilized and eliminated over a longer time period.

59. Depuration can be accelerated by an increase in **metabolic rate**. The metabolic rate of aquatic organisms increases with water temperature, and is accompanied by an increased rate of respiration (oxygen uptake). Rates of oxygen uptake closely parallel rates of contaminant uptake from water in aquatic species. Because metabolic rate influences both contaminant uptake and depuration, the net effect on bioaccumulation depends on whether the uptake or the depuration process is favored by the particular environmental circumstances. In the absence of external contamination, for example, the elimination of previously bioaccumulated contaminants is accelerated by an increase in metabolic rate. Conversely, if rising water temperatures happen to coincide with increased contaminant input to the water, the subsequent increases in organism metabolic rates tend to enhance contaminant bioaccumulation.

60. Metabolism or **biotransformation** is the process by which exogenous (foreign) chemical substances are enzymatically oxidized, reduced, cleaved, rearranged, or conjugated to form new compounds (metabolites) within the metabolically active organs of biota. The metabolites are usually more water soluble than the original chemicals and thus are more easily excreted. Therefore, the effect of biotransformation is generally to reduce the amount of unchanged chemical that is bioaccumulated by the organism. However, in some cases the metabolites may themselves be bioaccumulated rather than excreted. For example, DDT is biotransformed by most aquatic organisms to the -dichloroethane (DDD) metabolite and then to the -dichloroethylene (DDE) metabolite, which is retained. Over time, DDT concentrations in these organisms diminish, while DDE concentrations increase.

61. Organisms differ in their ability to biotransform foreign chemicals. Fishes, for example, generally metabolize chemicals more readily than do invertebrates, and mammals have greater biotransformation capability than fishes. Biotransformation ability also depends upon the class of chemicals involved. Fishes can metabolize PAHs, for instance, while bivalves and some amphipods accumulate these compounds because they lack the enzyme systems necessary to metabolize and eliminate PAHs. While some PAHs are acutely toxic, the metabolites of some PAHs are linked with chronic toxicity, such as carcinogenicity. The diol epoxide metabolite of benzo[a]pyrene, for example, is carcinogenic whereas the parent compound is not.

62. The **mixed-function oxidases** (MFOs) are the intracellular enzyme systems that function in the metabolism of foreign organic chemicals as well

as endogenous (biological) compounds such as steroids. Several types of MFOs are found in the metabolically active organs (e.g., liver) of all vertebrates, including fishes, and many invertebrates. In fishes and aquatic invertebrates, the most highly developed MFO systems are those that catalyze the biotransformation of planar, aromatic, lipid-soluble chemicals like the PAHs into more water-soluble compounds, thus facilitating their elimination. MFO systems are inducible (i.e., their biosynthesis is stimulated) by chemicals such as PAHs, dioxin, and some of the PCB congeners. Exposure to these chemicals induces appropriate MFOs for the detoxication of the chemicals. Induced organisms subsequently exposed to the same or chemically similar compounds are able to eliminate the chemicals more rapidly and bioaccumulate to lower levels than organisms not previously exposed to those chemicals (Buhler and Williams 1988; Kleinow, Melancon, and Lech 1987; Landrum 1982; Reichert, Le Eberhart, and Varanasi 1985; Stegeman 1985; Varanasi et al. 1985).

63. **Metallothioneins** are another class of biological compounds that are inducible. Metallothioneins are low molecular weight, sulfur-containing proteins in the kidneys, liver, gills, and digestive organs of most aquatic organisms. They bind with and function in regulating the metabolism of essential trace metals, and they also provide some protection against the toxic effects of metals such as copper, cadmium, zinc, and mercury. Low-level exposure of aquatic organisms to certain metal ions can produce a tolerance to the toxic effects of those metals through the induction of metallothioneins. This can result in an increased capability to bioaccumulate metals before the onset of toxic effects (Hamilton and Mehrle 1986; Harrison et al. 1987; Jenkins and Mason 1988; Klaverkamp and Duncan 1987; Viarengo et al. 1985).

64. **Lipids** are the storage compartment for hydrophobic chemicals in biota. Lipids are endogenous substances that are insoluble in water. They include structural substances such as phosphatides in cell membranes, substances such as steroids and carotenoids that are involved in various biochemical reactions, and the fats and waxes. Fats constitute reserve energy stores for organisms. Storage lipids composed primarily of fats have the greatest ability to contain hydrophobic chemicals. In general, the higher the total lipid content of an organism, the greater its capacity for bioaccumulation of hydrophobic chemicals (Schneider 1982).

65. The total lipid content of an aquatic organism (or in some cases the lipid content of specific tissues) is now frequently used as a basis for normalizing the concentrations of neutral organic contaminants found in

organisms (Geyer et al. 1982; Lundsford and Blem 1982; Boryslawskyj et al. 1988). Just as normalizing contaminant concentrations in sediment means dividing those concentrations by the decimal fraction TOC, lipid normalization refers to dividing wet weight tissue concentrations of contaminants in an organism by the decimal fraction lipid content of that organism. Normalization makes it possible to compare bioaccumulation among different species. Table 3 illustrates the effect of normalizing contaminant concentrations, using hypothetical data, for some example aquatic species having quite different lipid contents.

66. From Table 3 one can see that if wet-weight tissue concentrations are the same (e.g., dioxin) or nearly the same (e.g., endrin), then lipid-normalized concentrations will decrease considerably with increasing lipid content. If wet-weight contaminant concentrations decrease as species lipid contents increase (e.g., benzo[a]pyrene), then lipid-normalized concentrations decrease even more dramatically. If wet-weight contaminant concentrations increase as species lipid contents increase (e.g., total PCBs), then lipid-normalized concentrations decrease or increase, depending upon the amount of increase in the wet-weight concentrations. If lipid-normalized concentrations are held the same as lipid contents increase (e.g., DDE), then wet-weight concentrations will increase.

Table 3

Wet-Weight and Lipid-Normalized Contaminant Concentrations for Some Example Aquatic Organisms

Contaminant	Concentration Basis, μg/g	Organism: Mussels 1% Lipid	Killifish 5% Lipid	Lake Trout 15% Lipid
DDE	Wet weight	0.035	0.175	0.525
	Lipid-normal.	3.5	3.5	3.5
Endrin	Wet weight	0.004	0.005	0.006
	Lipid-normal.	0.40	0.10	0.040
Benzo[a]pyrene	Wet weight	1.8	0.18	0.018
	Lipid-normal.	180.0	3.6	0.12
Total PCBs	Wet weight	0.56	2.3	17.0
	Lipid-normal.	56.0	46.0	113.3
Dioxin	Wet weight	0.0001	0.0001	0.0001
	Lipid-normal.	0.01	0.002	0.0007

67. Unfortunately, analytical procedures for lipids in biota have not yet been standardized for environmental samples. Total lipids are often measured gravimetrically as the residue after evaporation of an organic solvent extract prepared for analysis of organic contaminants in aquatic biota. The most widely accepted lipid quantification method is that of Bligh and Dyer (1959). However, the Bligh-Dyer method may overestimate the amount of lipid that is actually available to absorb neutral organic chemicals. Rubinstein et al. (1987) found the Bligh-Dyer method cumbersome, and proposed using an aliquot of sample before the final clean-up stage, for a gravimetric determination of total lipids. This method is easier, more cost-effective, and more direct than the Bligh-Dyer method, and also may give an estimate that is closer to the amount of lipid actually available for partitioning of neutral organic chemicals. De Boer (1988) compared a number of solvent extraction systems with the Bligh-Dyer method and found 10 percent acetone/hexane to be an acceptable alternative that also left the sample ready for subsequent determination of PCBs. Brannon et al. (1990) used the 10-percent acetone/hexane extraction method in conjunction with a radio-labeled PCB congener in clam tissue to simultaneously extract PCB and lipid, weigh the lipid, and subsequently determine the amount of PCB in the lipid fraction of the clams.

68. An additional problem with lipid normalization is that a constant average lipid percent is generally used as the normalization basis for a given species; however, lipid content may vary considerably with season (Bierman 1990) and with age of the organism. Lake trout, for example, increase in lipid content from about 7 percent at age 3 to 5 years, to about 16 percent at age 7 to 10 years (Thomann and Connolly 1984). These variations have several implications for bioaccumulation. Partitioning of organic chemicals from water into lipids may result in age-dependent increases in bioaccumulation as lipid content increases, or in seasonal changes in bioaccumulation as lipid content changes in response to food supplies or spawning. Lipid-normalized chemical concentrations may increase in the spring, for example, as lipids are produced in preparation for spawning, and then decline dramatically in summer or fall following release of lipoidal gametes or subsequent lipid metabolism (Lunsford and Blem 1982).

Kinetics of Uptake and Elimination

69. This paper earlier described uptake and elimination of chemicals by an organism as kinetic (rate-influencing) processes for chemical transfer in the aquatic environment. This section will focus on how rates of uptake and elimination can be used to project maximum achieved bioaccumulation, i.e., the amount of chemical an organism would have accumulated in its tissues at steady state. Part III of this paper will describe how to use bioaccumulation testing to project steady-state bioaccumulation. Part III also describes ways to calculate maximum theoretical bioaccumulation potential for neutral organic contaminants in organisms of interest. This step would normally be done before any bioaccumulation testing and would help to determine the need for such testing.

70. Uptake and elimination of chemicals by an organism are processes that occur simultaneously. Nevertheless, for bioaccumulation to occur, a chemical must be taken up much faster than it can be eliminated. In other words, the uptake rate constant (k_1) will be much greater than the elimination rate constant (k_2). The difference between the rates of uptake and elimination determines the magnitude of bioaccumulation.

71. The kinetics of uptake and elimination are considered to be **first order**. In first-order eliminations, the amount of chemical in an organism decreases at a rate that is proportional to the amount remaining. As a consequence, the amount of chemical that is eliminated changes constantly depending on the amount of chemical that is left behind. The **half-life** of the chemical in the organism is the amount of time for half of the remaining chemical to be eliminated, and is constant regardless of the concentration in the organism.

72. To describe the kinetics of bioaccumulation, a one-compartment open model is most commonly used (Figure 4). In this model, all processes of elimination from the organism are described by the single elimination rate constant k_2. C_T is the chemical concentration in the organism, typically expressed in units such as micrograms per gram (μg/g) or milligrams per kilogram (mg/kg) (= parts per million, ppm), or nanograms per gram (ng/g) (= parts per billion, ppb). C_w is the chemical concentration in water, often expressed as micrograms per liter (μg/ℓ) (= ppb) or nanograms per liter (ng/ℓ) (=parts per trillion, pptr). The rate constant for chemical uptake from water (k_1), and k_2 are expressed in units of reciprocal time, such as per hour (h^{-1}) or per day (d^{-1}). The momentary rate of change of chemical concentration in the

organism is expressed by the differential equation describing this one-compartment model:

$$dC_T/dt = k_1C_w - k_2C_T \quad (4)$$

where t = time. The model is called one-compartment because it considers the organism to be a single, unified compartment. The integrated form of the one-compartment model equation is:

$$C_T = ((k_1C_w)/k_2)(1 - e^{-k_2t}) \quad (5)$$

As time t approaches infinity, the term e^{-k_2t} approaches zero and C_T becomes C_{ss}, the steady-state concentration of chemical in the organism:

$$C_{ss} = (k_1C_w)/k_2 \quad (6)$$

Finally, the ratio of the two rate constants is the bioconcentration factor K_B (assuming water is the only route of chemical exposure to the organism):

$$K_B = k_1/k_2 = C_{ss}/C_w \quad (7)$$

This is a simplistic model that makes many assumptions but generally gives a reasonable approximation of C_{ss}. More complex models have been derived that consider two or more compartments within the organism, each with its own uptake and elimination rate constants (Karara and Hayton 1984; O'Connor and Pizza 1987). Contaminant uptake from sources other than water (e.g., food or sediment) can also be factored into bioaccumulation models (Connolly and Pedersen 1988; Landrum 1988; Norstrom, McKinnon, and deFreitas (1976); Thomann 1981, 1989).

73. Part III of this paper describes how the first-order, one-compartment kinetic model can be used with bioaccumulation testing to project steady-state concentrations of chemical contaminants in aquatic organisms.

PART III: ASSESSMENT OF BIOACCUMULATION

Environmental Assessment of Sediments

74. Public laws regulating dredged material disposal (Section 404 of the Clean Water Act and Section 103 of the Ocean Dumping Act) require ecological evaluation prior to disposal of the material. This may include an assessment of the potential for bioaccumulation of toxic substances associated with dredged sediments. Part I of this paper sought to familiarize the reader with the concepts and terminology of bioaccumulation, and with the fundamental physical, biological, and chemical factors affecting bioaccumulation. The portion of this paper that follows describes the assessment of bioaccumulation under a four-tiered testing approach for dredged material evaluation (US Environmental Protection Agency (USEPA) 1990). The derivation and step-by-step procedures for calculating both the potential for bioaccumulation (in Tier II) and the projected achievable bioaccumulation (in Tiers III and IV) are included.

The Tiered Testing Approach

75. The Corps and the USEPA have developed a procedure for evaluating proposed dredged material disposal (USEPA 1990). This procedure consists of a national, comprehensive, tiered testing approach for evaluation of aquatic disposal of dredged material. Each of the four tiers in this approach is based on a "reason to believe" that there is potential for unacceptable adverse effects on the environment from aquatic disposal. The tiered testing approach is described in detail for ecological evaluation of proposed discharge of dredged material into ocean waters (USEPA 1990). Briefly, the approach includes initial evaluation of existing information (Tier I), bulk sediment inventory and elutriate analysis (Tier II), and biological testing for acute toxicity and bioaccumulation in evaluating water column impacts and deposited sediment impacts (Tiers III and IV). Flowcharts outlining the entire tiered testing approach are illustrated in Figures 3.1, 3.2, and 3.3 of USEPA (1990). The discussions that follow herein will focus only on the evaluations in each tier that relate to bioaccumulation.

76. **Tier I** involves the evaluation of existing information in the initial determination of "reason to believe" that there is potential for

unacceptable adverse impacts from aquatic disposal of dredged material. The "existing information" can include sediment grain size, historical bioaccumulation data, proximity of the dredging site to known contaminant sources such as spills or discharges, proximity of the disposal site to critical or sensitive natural resources, etc. Tier I does not require any new testing. In many cases, Tier I information is sufficient to determine definitively that the dredged material either is acceptable for ocean disposal, or is not acceptable for ocean disposal without management action. If Tier I information is not sufficient for this determination, then further evaluation is required in Tiers II, III, and/or IV.

77. **Tier II** bioaccumulation evaluation involves the determination of potential for benthic impacts from the bioaccumulation of contaminants in the dredged material. A maximum theoretical bioaccumulation potential can be calculated for neutral organic contaminants. This calculation requires analysis of neutral organic contaminant concentrations and TOC in the dredged material and an appropriate reference sediment. The background and methodology for this calculation are described in detail in later sections of this paper. If bioaccumulation potential from the dredged material is less than or equal to that from the reference sediment, then no further testing for neutral organic contaminant bioaccumulation is required. Bioaccumulation testing in Tiers III and/or IV is required if (a) bioaccumulation potential from the dredged material is greater than that from the reference sediment or greater than US Food and Drug Administration (FDA) limits, (b) if contaminants other than neutral organics (such as metals) are of concern, or (c) if the information generated in Tier II is not adequate for decision making.

78. **Tier III** bioaccumulation evaluation requires biological testing. This is known as single-time-point bioaccumulation testing because organism samples for contaminant analysis are collected only once, at the end of the exposure period. If contaminant concentrations in organisms exposed to the dredged material for 10 days (metals only) or 28 days (organic or organometallic compounds) exceed FDA limits, then ocean disposal without management action is unacceptable. If organisms exposed to the dredged material for 10 or 28 days bioaccumulate significantly higher concentrations of contaminants than organisms exposed to an appropriate reference sediment for the same length of time, then Tier IV evaluation is required.

79. **Tier IV** bioaccumulation evaluation involves the analysis of tissues of biota collected in the field or time-sequenced bioaccumulation testing in

the laboratory to estimate steady-state bioaccumulation. Field assessment is limited to maintenance dredging involving sediments that are demonstrably unchanged since the last dredging operation. The disposal site involved must also be the same as previously used, or must be physically and biologically similar to the previous site. Field assessments are limited in usefulness by these constraints.

80. Laboratory assessments of steady-state bioaccumulation involve collecting a sequence of samples over time from organisms exposed to the dredged material and from organisms exposed to an appropriate reference sediment. Each sample should consist of enough individuals to provide sufficient biomass for chemical analysis. Analysis of these samples for contaminant concentrations allows generation of uptake curves and calculation of uptake and depuration rate constants, from which steady-state bioaccumulation can be determined. If steady-state bioaccumulation in organisms exposed to the dredged material exceeds FDA action levels or other criteria, then ocean disposal without management action is unacceptable. If steady-state bioaccumulation in organisms exposed to the dredged material exceeds that in organisms exposed to the reference sediment, then case-specific criteria that have been developed for the particular disposal operation must be assessed to determine whether ocean disposal without management action is or is not acceptable.

81. Species selection, laboratory procedures, and statistical analyses for Tier III and Tier IV bioaccumulation tests are described in USEPA (1990). Methods for calculating steady-state bioaccumulation are detailed later in this paper.

Bioaccumulation Potential (Tier II)

82. Methods for estimating bioaccumulation potential have been developed only for neutral organic chemicals, such as PCBs, DDT and its metabolites, PAHs, dioxins, and furans. Thus, discussions in this paper concerning Tier II calculations will be restricted to neutral organic chemicals. Usually, this class of chemicals includes the contaminants of greatest concern in dredged material.

83. In conducting environmental assessments of dredged material slated for aquatic disposal, one is ultimately concerned about the potential for unacceptable adverse impacts, both acute and chronic, on biota. Measuring (or estimating) bioaccumulation is one way of evaluating the potential for adverse

chronic effects on biota, because contaminants must be taken up or retained by biota in order to have chronic effects. Of course there are exceptions to this assumption. Compounds like PAHs that are readily metabolized by certain organisms, such as fishes, may show little bioaccumulation, yet some of the metabolites may be carcinogenic. Thus, the determination of bioaccumulation has most utility for evaluating potential adverse effects from neutral organic chemicals such as PCBs, dioxins, and furans, which undergo relatively little metabolic transformation in aquatic organisms.

Estimating bioaccumulation
using partition coefficients

84. Part I of this paper discussed how bioaccumulation is influenced by thermodynamic properties of the aquatic environment, such as fugacity and equilibrium partitioning. Water is one of the two compartments used to describe equilibrium partitioning in terms of partition coefficients. However, in assessing bioaccumulation potential from sediment, the concentration of chemical in water is not of central interest. It would be much simpler to use the concentration of chemical in sediment to predict the equilibrium concentration in biota. Nevertheless, the transfer of chemical from sediment to biota is generally mediated by water. Therefore, the relationships with water represented by the partition coefficients K_{oc} and K_B can be used to calculate equilibrium concentrations between sediment and water, and then between water and biota. These relationships are illustrated in Figure 8. A later section will describe an easier method that eliminates the need for water concentrations as an intermediate step in the calculation of bioaccumulation potential.

85. Calculation of bioaccumulation potential using partition coefficients is described as follows, using DDT as an example. Assume that the concentration of DDT in sediment (C_s) is 1.0 ppm, and the total organic carbon content of the sediment is 3 percent (TOC = 0.03). To determine the concentration of DDT in an aquatic organism at equilibrium with the sediment, it is first necessary to normalize the sediment concentration of DDT on TOC to obtain C_s^{oc}:

$$C_s^{oc} = C_s/TOC \qquad (8)$$
$$= 1.0/0.03 = 33.33 \text{ ppm}$$

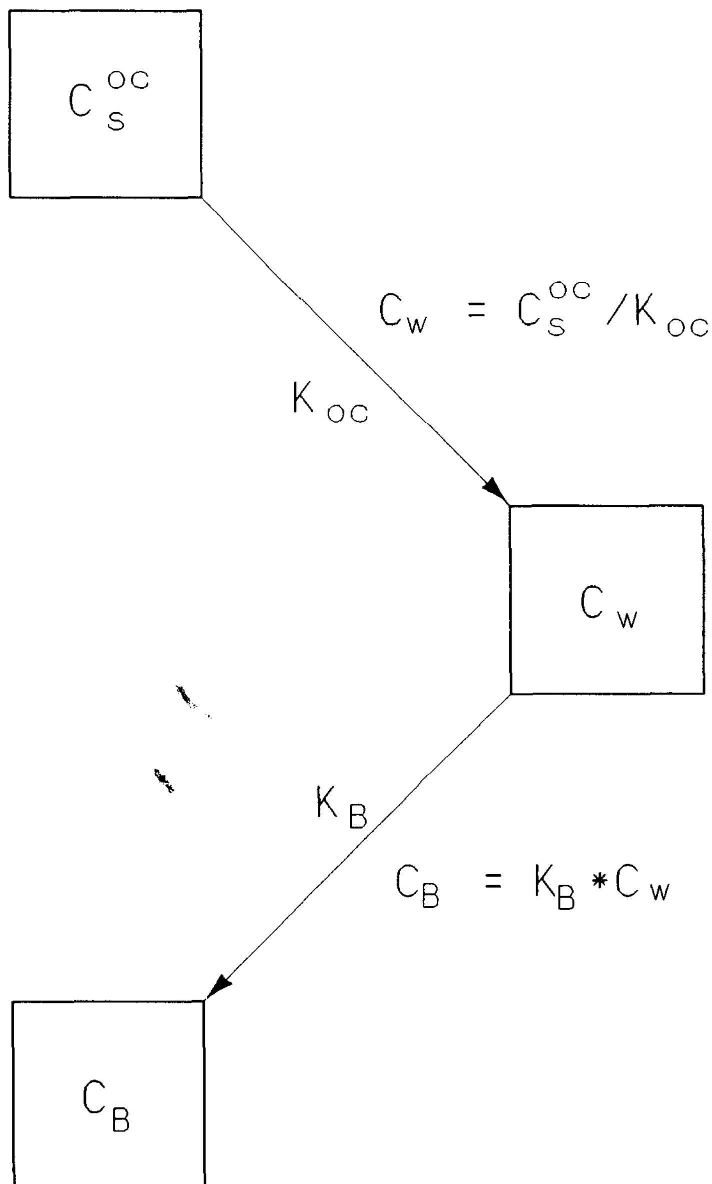


Figure 8. Calculation of bioaccumulation C_B using chemical concentration in sediment organic carbon (C_s^{oc}) along with partition coefficients K_{oc} and K_B

Reported partition coefficients for DDT include:

log K_{ow} = 5.75, 6.19 (Veith, DeFoe, and Bergstedt 1979; Chiou et al. 1977)

log K_{oc} = 5.38 (Karickhoff 1981)

log K_B = 4.47 (Veith, DeFoe, and Bergstedt 1979)

The next step involves using the concentration of DDT in sediment organic carbon (C_s^{oc}) and the sediment organic carbon:water partition coefficient (K_{oc}) to calculate the concentration of DDT in water (C_w) (Figure 8):

$$C_w = C_s^{oc}/K_{oc} \quad (9)$$
$$= 33.33/\text{antilog } 5.38$$
$$= 0.000139 \text{ ppm}$$

The third step uses the calculated concentration of DDT in water (C_w) and the bioconcentration factor (K_B) for DDT to calculate the concentration of DDT in the organism (C_B) (Figure 8):

$$C_B = K_B \times C_w \quad (10)$$
$$= \text{antilog } 4.47 \times 0.000139$$
$$= 4.1 \text{ ppm}$$

Thus, when the organism is in equilibrium with the sediment, it could bioaccumulate 4.1 ppm DDT, assuming that sediment is the only source of contaminant to the organism and that all bioaccumulation from sediment is mediated through the water. C_B in this case is a whole-body concentration.

86. Now, suppose only log K_{ow} is known for DDT, and not log K_{oc} or log K_B. (Octanol:water partition coefficients are available for many more compounds than are sediment organic carbon:water partition coefficients or bioconcentration factors.) In this case, published estimator equations for neutral organic chemicals can be used to estimate log K_{oc} and log K_B from log K_{ow}:

$$\log K_{oc} = 0.989 \log K_{ow} - 0.346, \qquad r^2 = 0.987 \quad (11)$$

(from Karickhoff 1981)

$$\log K_B^{lipid} = 0.980 \log K_{ow} - 0.063, \qquad r^2 = 0.982 \quad (12)$$

(from Könemann and van Leeuwen 1980)

Equation 12 gives a bioconcentration factor for the lipids of an organism. Using the first log K_{ow} value for DDT above, 5.75, in Equations 11 and 12, the estimated log K_{oc} for DDT = 5.34 and log K_B^{lipid} for DDT = 5.57. Calculations

for Equations 9 and 10 then provide the concentration of chemical in the organism lipids at equilibrium (C_B^{lipid}):

$$C_w = 33.33/\text{antilog } 5.34$$
$$= 0.000152 \text{ ppm}$$

$$C_B^{lipid} = \text{antilog } 5.57 \times 0.000152$$
$$= 56.5 \text{ ppm}$$

Thus, an organism in equilibrium with the sediment would have 56.5 ppm DDT in its lipids. To convert this concentration to whole body,

$$C_B \text{ (whole body)} = C_B^{lipid} \times fL \quad (13)$$

where fL = decimal fraction lipid. If a particular species of interest has a mean lipid concentration of 6 percent, then,

$$C_B \text{ (whole body)} = 56.5 \text{ ppm} \times 0.06$$
$$= 3.4 \text{ ppm}$$

for that species. Repeating the above calculations using the second log K_{ow} given for DDT, 6.19, produces a slightly different estimate of bioaccumulation for the species of interest. From Equations 11 and 12, estimated log K_{oc} for DDT = 5.78 and log K_B^{lipid} for DDT = 6.00. Then,

$$C_w = 33.33/\text{antilog } 5.78 = 0.0000553 \text{ ppm}$$
$$C_B^{lipid} = \text{antilog } 6.00 \times 0.0000553 = 55.3 \text{ ppm}$$
$$C_B \text{ (whole body)} = 55.3 \text{ ppm} \times 0.06 = 3.32 \text{ ppm}$$

Note that there is almost a three-fold difference in magnitude between the two reported octanol:water partition coefficients for DDT (antilog 5.75 = 562,341 and antilog 6.19 = 1,548,817). However, the equilibrium tissue concentrations for DDT calculated from these two log K_{ow} values (3.4 ppm from log K_{ow} = 5.57, and 3.32 ppm from log K_{ow} = 6.19) are nearly the same. It is not particularly important what log K_{ow} values are used; as long as the same pair of estimator equations are used, the result will be much the same. The accuracy of the estimation is dependent on the quality of the estimator equations and not on

the accuracy of the particular partition coefficient. In the next section, a method that is based on this observation and does away with the need for partition coefficients will be discussed.

87. Estimating bioaccumulation potential from sediments using partition coefficients, although relatively simple, has several disadvantages. First, three partition coefficients are involved, none of which can be measured without error. Therefore, the estimate of bioaccumulation potential derived from these partition coefficients is associated with an unknown cumulative error. Second, it is often very difficult to find reported K_{oc} and K_B values for a particular chemical that are appropriate for the organism and exposure conditions of interest. These parameters must then be estimated from log K_{ow}, which contributes an additional source of error. Finally, measurements of highly hydrophobic chemical concentrations in water are analytically difficult and generate results that are often suspect.

88. Bioaccumulation potential estimations that do not rely on measurements of hydrophobic chemicals in water can be more accurate because less cumulative error may exist. One such approach, **theoretical (thermodynamic) bioaccumulation potential (TBP)**, is even simpler than the partition coefficient calculations described above. The TBP method is presented in the next section.

TBP: A simpler approach for
estimating bioaccumulation potential

89. TBP is an *a priori* estimate of the equilibrium concentration of a chemical in the tissues of an organism exposed to sediment containing that chemical. TBP requires knowledge only of the concentration of chemical in the sediment, the organic carbon content of the sediment, and the lipid content of the organism. As stressed above in the general discussion of prediction methods for bioaccumulation potential, TBP is suitable only for neutral organic chemicals. TBP is not an empirical measurement or an absolute maximum value for bioaccumulation.

90. TBP derives from equilibrium partitioning theory and is based on the premise that organic solvents have greater fugacity capacity or containing ability than water for neutral organic chemicals. This relationship is illustrated by the solubilities given in Table 4 for neutral organic chemicals in organic solvents as compared with their solubility in water. The solubility of organic chemicals in organic solvents is near unity (10^0 to 10^{-1}), whereas

the solubility of the same chemicals in water is on the order of parts per million to parts per trillion (10^{-6} to 10^{-12}).

Table 4

Solubilities of Organic Solutes in Organic Solvents or in Water, g/ml

Organic Solute	Molecular Weight	Solvent: Toluene or Benzene	Olive Oil or Peanut Oil	$CHCl_3$	CCl_4	Water
Naphthalene	128.16	0.29	0.13	0.5	0.5	8.80×10^{-7}
Phenanthrene	178.22	0.42			0.42	1.06×10^{-9}
Lindane	290.85	0.29		0.24		1.70×10^{-8}
DDT	354.5	0.78	0.11		0.45	3.10×10^{-12}

Sources of data: Merck Index (1983), Verschueren (1983)

91. Both organic carbon in sediments and lipids in biota behave toward organic chemicals as though they were organic solvents. Therefore, neutral organic chemicals will tend to partition into the organic carbon of sediments and the lipids of organisms rather than remain in the aqueous phases of these two compartments. Even though desorption of the neutral organic chemical from sediment to organism or vice versa occurs through the water, the concentration of the chemical in water can be ignored since it will be negligible compared to the concentrations in sediment organic carbon and organism lipid. Thus, TBP depends on the assumption that all of the neutral organic chemical in the sediment organic carbon can move into the lipids of an organism that has been exposed to the sediment long enough for equilibrium to occur.

The preference factor

92. TBP estimates the equilibrium concentration of a neutral organic chemical (normalized on lipid content) in biota directly from the concentration of that chemical (normalized on organic carbon content, C_s^{oc}) in sediment. TBP incorporates a unitless constant called the **preference factor** pf (Figure 9), determined as follows:

$$pf = C_B^{lipid}/C_s^{oc} \quad (14)$$

The preference factor is a measure of the "preference" of neutral organic chemicals for lipid over sediment organic carbon. A preference factor greater

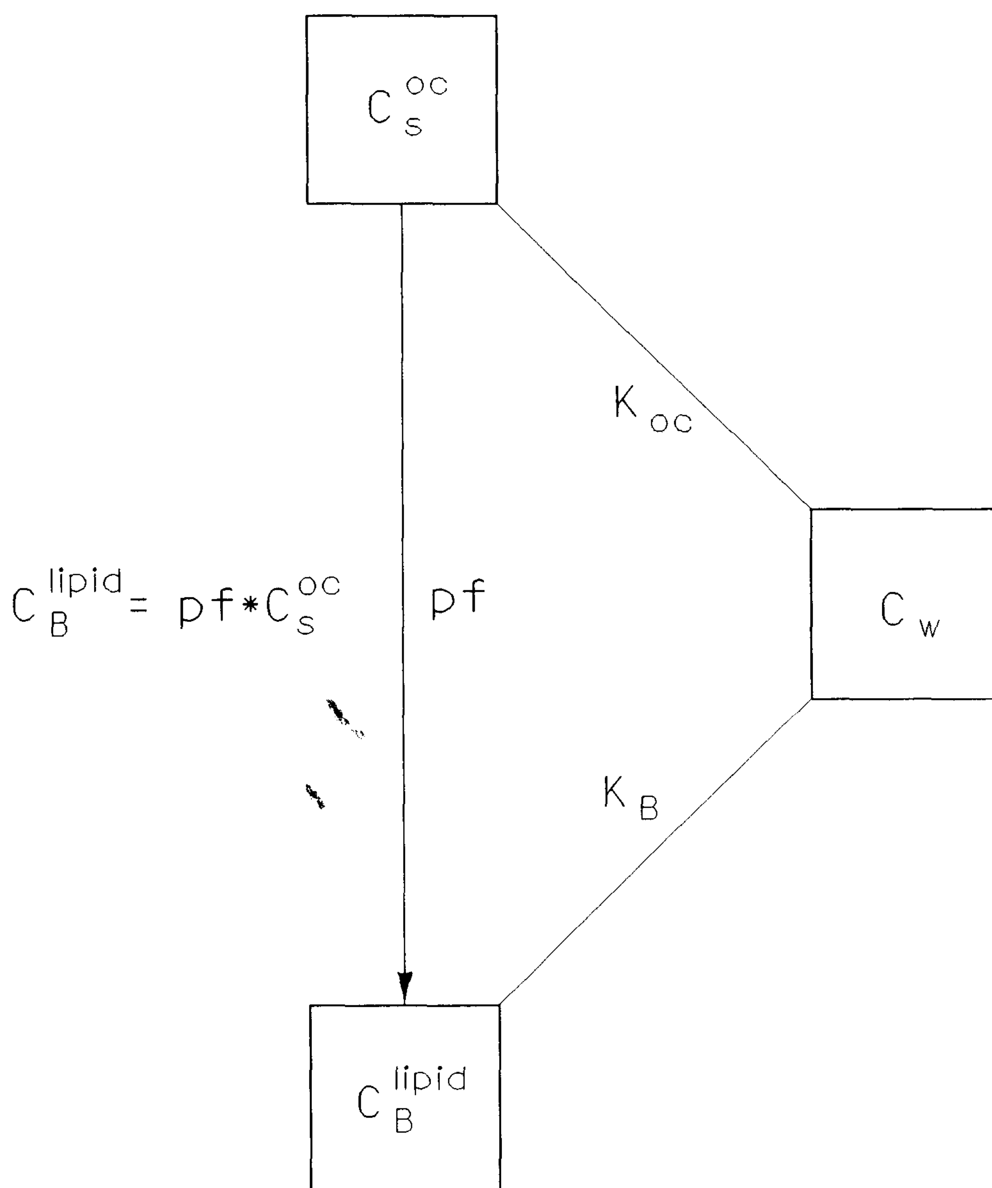


Figure 9. Calculation of neutral organic chemical concentration in organism lipid (C_B^{lipid}) directly from the concentration in sediment organic carbon (C_s^{oc}) using the preference factor (pf)

than 1 indicates that neutral organic chemicals "prefer" lipid to sediment organic carbon. For example, if pf = 2, then the concentration of a neutral organic chemical in lipid at equilibrium will be twice that in sediment organic carbon. The preference factor has also been called the accumulation factor or AF (Ferraro et al. 1990), the apparent preference factor or APF (McElroy and Means 1988), and the partitioning factor or PF (Lake, Rubinstein, and Pavignano 1987). Empirically or semi-empirically determined values for these factors have ranged from less than 1 to 4 or more. This paper will

first describe a semi-empirical derivation of pf (McFarland and Clarke 1986), and then discuss some empirical values of preference factors determined by these and other investigators.

93. McFarland and Clarke (1986) calculated pf from the combined data sets of Karickhoff (1981) and Könemann and van Leeuwen (1980) (Figure 10). The data of Karickhoff regressed empirical values of log K_{oc} on log K_{ow} for a group of five PAHs ranging from benzene (1 ring) to pyrene (4 rings) (Equation 11). The data of Könemann and van Leeuwen similarly regressed lipid-normalized log K_B on log K_{ow} for a group of five chlorobenzenes in guppies (Equation 12). This data set originally included a value for a sixth chlorobenzene having log $K_{ow} > 6$, which was deleted for the sake of the pf calculations so that the regression would remain in the linear portion of its range (see discussion of hydrophobicity in paragraph 17, and Figure 5).

94. The difference between the two essentially parallel regression lines in Figure 10 can be considered an approximation of the difference in activities (or fugacity capacities) of neutral organic chemicals in the two phases:lipid and sediment organic carbon. Thus, the difference can be used to determine the "preference" of neutral organic chemicals for one phase as opposed to the other. The two regression equations can be used to estimate log K_{oc} and log K_B at the arithmetic mean K_{ow} (the log of the arithmetic mean K_{ow} of the combined data sets = 4.923). Lower and upper 95-percent confidence limits for the estimates are given in parentheses:

$$\log K_{oc} = 0.989 \times 4.923 - 0.346 = 4.523 \quad (4.412,\ 4.634)$$

$$\log K_B = 0.980 \times 4.923 - 0.063 = 4.762 \quad (4.581,\ 4.943)$$

The antilog of the difference between these two estimates is pf:*

$$\begin{aligned} pf &= \text{antilog}\ (4.762 - 4.523) \\ &= \text{antilog}\ 0.239 = 1.73 \quad (1.48,\ 2.04) \end{aligned}$$

* pf was originally calculated as 1.92 (or 1/0.52), the antilog of the difference in the y-intercepts of Equations 11 and 12 (McFarland 1984; McFarland and Clarke 1986). However, the y-intercepts lie outside the range of the data for these regression lines; therefore, taking the difference in the estimated y values on the regression lines at the mean of the combined data is a more appropriate derivation for pf.

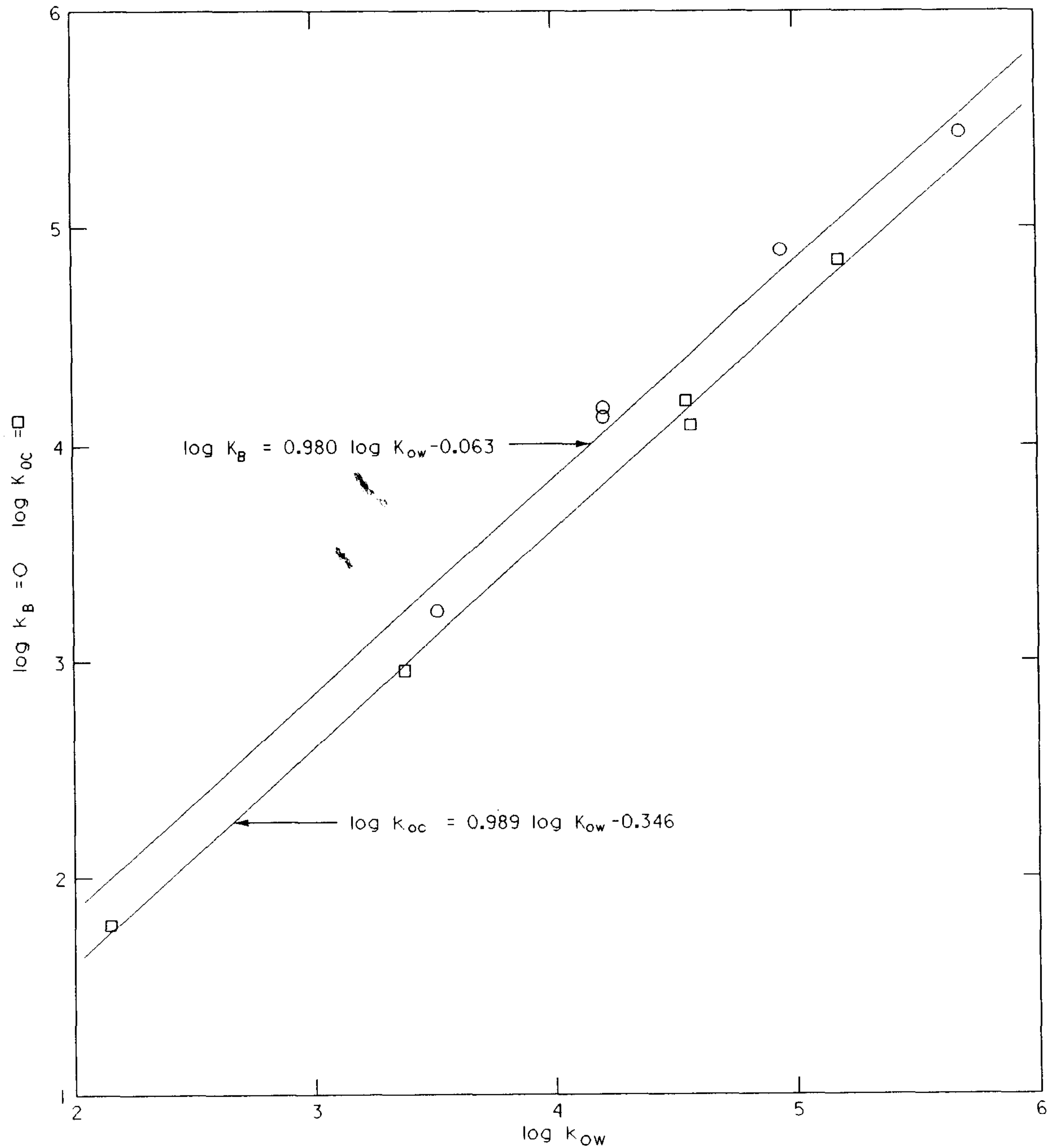


Figure 10. Linear regressions showing the relationship between log K_{ow} and log K_B (data of Könemann and van Leeuwen 1980), and between log K_{ow} and log K_{oc} (data of Karickhoff 1981)

The confidence limits in parentheses are antilogs of the difference between the lower confidence limits for the log K_{oc} and K_B estimates, and between the upper confidence limits for the two estimates, i.e.:

$$\text{lower 95\% confidence limit for pf} = \text{antilog } (4.581 - 4.412) = \text{antilog } 0.169 = 1.48$$

$$\text{upper 95\% confidence limit for pf} = \text{antilog } (4.943 - 4.634) = \text{antilog } 0.309 = 2.04$$

Since the calculation of pf involved the subtraction of log K_{oc} from log K_B, pf is therefore the preference of neutral organic chemicals for lipid over sediment organic carbon. A pf for sediment organic carbon over lipid would be the reciprocal of 1.73, or 0.58. Either way, the pf indicates that neutral organic chemicals have a slightly greater tendency to partition into lipid than into sediment organic carbon.

Calculation of TBP

95. TBP is calculated as follows:

$$\text{TBP} = \text{pf} \times (C_s/\text{TOC}) \times \text{fL} \quad (15)$$

where

TBP is expressed as whole-body (wet) weight in the same units as C_s

pf = preference factor for organism lipid over sediment organic carbon

C_s = concentration of chemical in sediment (usually dry weight)

TOC = decimal fraction (or percent) total organic carbon in sediment

fL = organism lipid expressed in the same units as TOC (decimal fraction or percent)

Recall the calculations of bioaccumulation potential given above for DDT. Now, calculating TBP for the same DDT situation, where C_s = 1.0 ppm, TOC = 0.03, fL = 0.06, and pf = 1.73 as derived above:

$$\text{TBP} = 1.73 \times (1.0/0.03) \times 0.06 = 3.46 \text{ ppm}$$

Substituting the pf lower and upper confidence limits for pf in Equation 15 results in a 95-percent confidence interval for the estimate of TBP:

lower 95% confidence limit for TBP = 1.48 × (1.0/0.03) × 0.06
= 2.96 ppm

upper 95% confidence limit for TBP = 2.04 × (1.0/0.03) × 0.06
= 4.08 ppm

The TBP confidence interval is a measure of the statistical variability around the preference factor, and does not include components for chemical analytical error or error in the original data for the determination of C_s, TOC, and fL.

96. The TBP value (3.46 ppm) for DDT is nearly the same as the values of bioaccumulation potential (3.4, 3.32) for DDT calculated above using partition coefficients. However, the TBP approach is simpler and may be more practical in many situations because it does not require knowledge of partition coefficient values in addition to sediment concentrations of the contaminants of interest.

Other values for pf

97. Various investigators have determined empirical values for the preference factor. Some of these values are equivalent to pf as defined above. Others are the reciprocal of pf, i.e., they indicate the "preference" of neutral organic chemicals for sediment organic carbon over lipid rather than vice versa. For the sake of comparison, all preference factors reported herein are expressed on the same basis as pf, i.e., the preference for lipid over sediment organic carbon.

98. Lake, Rubinstein, and Pavignano (1987) reported PFs for a number of laboratory- and field-exposed organisms. The mussel *Mytilus edulis* exposed in the laboratory to heavily contaminated sediments had PFs of 0.5 and 2.5; the same species exposed to background contamination in unfiltered seawater produced a PF of 3.23. Polychaetes and clams exposed in the field to sediments having low levels of contaminants resulted in mean PFs ranging from 2 to 10 (overall mean = 3.92). Mean PFs determined for specific chlorinated compounds (including DDD and chlordane) in two deposit feeders, the polychaete *Nepthys incisa* and the clam *Yoldia limatula*, exposed in the field to sediments having low-level contamination, ranged from 3.33 to 5.88 (overall mean = 4.36).

99. Rubinstein et al. (1987) exposed *N. incisa*, *Y. limatula*, another polychaete, *Nereis virens*, and another clam, *Macoma nasuta*, to several sediments in the laboratory for 45 to 60 days. The sediments ranged from relatively uncontaminated to highly contaminated. Accumulation factors (AFs) were calculated for Aroclor 1254 and 13 individual PCB congeners in 110 samples

(923 AFs total). The least-contaminated sediment tended to yield the highest AFs. The overall mean AF for Aroclor 1254 was 5.76. AFs for individual PCB congeners having seven or more chlorine atoms tended to be lower than AFs for congeners containing less than seven chlorine atoms, perhaps indicative of steric hindrance to bioaccumulation, insufficient time to reach steady state for the most highly chlorinated PCBs, or reduced bioavailability due to strong sorption to sediments. Mean AFs for the individual PCB congeners ranged from 1.53 to 7.18.

100. McElroy and Means (1988) exposed *N. incisa* and *Y. limatula* to two sediments containing hexachlorobiphenyls, and determined apparent preference factors (APFs). The APFs, which reached a constant value within 20 days, averaged 0.43 and 0.21 for *Nephtys*, and 1.68 and 0.86 for *Yoldia*. The authors called these preference factors "apparent" because "true" preference factors require thermodynamic equilibrium in the exposure system, which they did not determine in their experiment. They attributed most of the variability in their APFs to variability in organism lipid content, and recommended that a single, standardized extraction method for determining lipid content be adopted.

101. Clarke, McFarland, and Dorkin (1988) suggested that an aquatic confined disposal facility (CDF) may represent an ecosystem in which contaminant partitioning is actually near equilibrium. Transport of water, sediment, and aquatic organisms between the CDF and its adjacent body of water is limited; thus, disturbances that disrupt equilibrium partitioning in the CDF may be minimal. The investigators determined pfs ranging from 1.48 to 4.45 (mean = 2.98) for crayfish and eight species of fishes collected inside the CDF, and suggested that the laboratory-derived pf of 1.73 may be too low for field situations involving contaminated sediments and associated biota at steady state.

102. Bierman (1988) determined accumulation factors (the ratio C_B^{lipid}/C_s^{oc}) for PCBs and PAHs in field-exposed organisms from the Great Lakes. Mean accumulation factors were 0.71 for PCBs in oligochaetes from Lake Ontario and the Detroit River, 5.87 for PAHs in Lake Erie oligochaetes and chironomid midges, and 3.14 for PAHs in the amphipod *Pontoporeia hoyi* from Lake Michigan. In another study, Bierman (1990) examined animal-sediment concentration ratios (log C_B^{lipid}/C_s^{oc}) from several field studies involving numerous chemicals and organisms in the Great Lakes. He found significant variations in these ratios among chemicals, species, and seasons, implying that accumulation factors are

not constant except perhaps for very homogeneous groups of chemicals and organisms where seasonal influences have been eliminated.

103. Ferraro et al. (1990) determined accumulation factors for 10 organic pollutants in clams (*Macoma nasuta*) exposed in the laboratory for 28 days to six field-contaminated sediments. Contaminants included DDE, DDD, the PCB mixture Aroclor 1254, two PCB congeners, and five PAHs. Mean AFs ranged from 0.05 to 2.8 (overall mean = 0.65), and tended to be highest for DDE and lowest for the PAHs. Ferraro et al. (in review) also reported mean AFs from the same exposures for 11 PCB congeners, ranging from 0.16 to 2.1 (overall mean = 0.79). Maximum individual AFs ranged from 1.02 to 5.42.

104. Pruell et al. (1990) determined AFs for several organisms exposed to sediment in the laboratory for 180 days (*Nereis*), 120 days (*Macoma*), and 28 days (the shrimp *Palaemonetes*). AFs for these species were, respectively, 0.505, 0.664, and 0.824 for total PCBs; 0.457, 0.684, and 0.843 for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Highest AFs occurred in *Macoma*, while the lowest occurred in *Nereis*.

105. Young, Mearns, and Gossett (in press) recently reanalyzed an extensive data set collected on DDE and PCB in flatfish in the Southern California Bight during the late 1970's. Fish and sediments were collected from an uncontaminated reference zone and from contaminated areas in the vicinity of sewage outfalls in the Palos Verdes Shelf. When AFs were calculated from either area they were remarkably similar to the theoretical value of 1.73 reported for pf by McFarland and Clarke (1986). For the reference area and the contaminated areas, respectively, the AFs for *p,p'*-DDE were 1.8 and 1.7 in muscle tissue and 3.4 and 2.0 in liver. For PCB (Aroclor 1254) the AFs were 1.3 and 0.96 in muscle and 2.7 and 1.4 in liver. The trend toward higher AFs for less contaminated sediments was consistent with the findings of others (Rubinstein et al. 1987; Clarke, McFarland, and Dorkin 1988).

106. The draft guidance for ecological evaluation of dredged material disposal (USEPA 1990) recommends using a preference factor of 4 in the calculation of TBP. Review of all accumulation/preference factors listed in the references above indicates that pf for field studies averaged 4, whereas pf for laboratory studies averaged ~3. For pf to be valid, bioaccumulation must be measured under steady-state conditions, which is difficult or impossible to determine in the field. Nevertheless, concern about the effects of bioaccumulation from dredged material disposal ultimately centers on aquatic organisms in their natural environment rather than in the laboratory, and so pf = 4 is

probably the more appropriate (and certainly the more environmentally conservative) value to use in TBP calculations.

107. Recalculating TBP for DDT, as in the previous exercise, using only pf = 4 (instead of 1.73) in Equation 15 yields:

$$\begin{aligned} TBP &= 4 \times (1.0/0.03) \times 0.06 \\ &= 8.0 \text{ ppm} \end{aligned}$$

This is a more conservative, and thus, more environmentally protective, value for TBP than the value of 3.46 obtained using pf = 1.73. The FDA action level for DDT is 5 ppm in the edible portions of fish and shellfish. Consider an aquatic species having a mean lipid content of 6 percent. TBP calculated using the lower pf would indicate that bioaccumulation of DDT should not be a problem in this species because the theoretical bioaccumulation potential (3.46 ppm) is less than the FDA action level. TBP calculated using the higher pf, however, would indicate that DDT bioaccumulation could be a problem in this species (TBP = 8.0 ppm) and that bioaccumulation testing (Tiers III or IV) would need to be done.

Limitations of TBP

108. Calculation of bioaccumulation potential requires, at minimum, knowledge of the sediment concentration of the contaminant of interest, the sediment TOC, and the fraction of lipid in the organism of interest. The TBP method is simple and straightforward because it requires only a preference factor in addition to these parameters. Nevertheless, TBP has some important limitations:

a. TBP assumes that sediment is the only source of contaminant to the organism. In particular, TBP does not take food chain uptake into account, nor is it adjusted for organism growth, both of which can be significant factors in the field (Thomann 1989).

b. TBP can be calculated only for neutral organic chemicals, and may be appropriate only for those chemicals in the log K_{ow} range of about 2 to 7.

c. TBP assumes that a single preference factor represents all combinations of organic chemicals, sediment organic carbon, and organism lipid. This is not necessarily true, but is considered a useful simplification.

d. TBP is a thermodynamic idealization that ignores kinetic constraints. In the real world, kinetic constraints exist both on bioavailability and on elimination of chemicals, and will

affect actual levels of bioaccumulation of chemicals from sediment.

e. TBP assumes that metabolism is not a factor in the bioaccumulation of neutral organic chemicals.

109. TBP is most appropriately used as a simple approximation to indicate the levels to which neutral organic chemicals in sediments may be bioaccumulated by organisms exposed to the sediments. TBP should be used in Tier II (perhaps in Tier I if knowledge of sediment contaminant concentrations, TOC, and organism lipid content is already available) of the dredged material evaluation process for aquatic disposal. In this way, TBP serves as a screening tool to help one decide from sediment chemistry data whether or not actual bioaccumulation testing (Tiers III or IV) should be conducted.

Bioaccumulation Testing (Tiers III and IV)

Tier III bioaccumulation testing

110. If Tier II TBP calculations indicate that any neutral organic contaminants in the dredged material are present in concentrations that could result in unacceptable bioaccumulation, then Tier III (or IV) bioaccumulation testing is conducted. Laboratory procedures for conducting these tests and analyzing the results are given in USEPA (1990) and in Lee et al. (1989).

111. In Tier III bioaccumulation testing, aquatic organisms are exposed to dredged material and to an appropriate reference sediment for 10 days (assessment of metals) or for 28 days (assessment of organic contaminants). At the end of the exposure period(s), replicate tissue samples are collected and analyzed for the contaminants of concern. The significance of contaminant bioaccumulation from the dredged material is determined by comparison with FDA limits, if available, or otherwise by statistical comparison with bioaccumulation from the reference sediment. However, statistically greater bioaccumulation from dredged material than from reference exposures as determined from a single time point only indicates a difference in bioavailability. In many cases, it is necessary to determine whether expected contaminant body burdens at steady state are high enough to be of concern. If one wishes to look at steady state tissue concentrations of sediment contaminants, then further calculations (Tier III) or sample analyses (Tier IV) are necessary.

How to estimate steady state
from the 28-day tissue concentration

112. For neutral organic chemicals, steady-state tissue concentrations (C_{ss}) can be estimated using mathematical relationships derived from the one-compartment bioaccumulation model (Equations 4 and 5). When only single-time-point bioaccumulation samples are available (e.g., the Tier III bioaccumulation tissue samples taken on day 28), estimation of steady state involves a five-step calculation procedure. The first step is to estimate the time to steady state for a given contaminant. This can be done using log K_{ow} (for chemicals in the log K_{ow} range 2.5 to 9.5) in a polynomial equation based on bioconcentration of chlorinated hydrocarbons and closely related compounds by fish (Connell and Hawker 1988):

$$\log t_{ss} = 6.9 \times 10^{-3}(\log K_{ow})^4 - 1.85 \times 10^{-1}(\log K_{ow})^3 + 1.65(\log K_{ow})^2 - 5.34(\log K_{ow}) + 5.93 \quad (16)$$

where t_{ss} is the time in days to 99 percent of steady state. Because the uptake curve in the one-compartment bioaccumulation model approaches steady state asymptotically (Figure 11), it is not possible to calculate 100 percent of steady state, and thus 99 percent of steady state may be considered equivalent to steady state. Equation 16 describes a curve (Figure 12) in which compounds of low hydrophobicity (log K_{ow} <3 to 4) reach steady state tissue concentrations in approximately 1.8 days. As log K_{ow} increases above 4, t_{ss} also increases to a maximum of 325 days (0.89 years) at log $K_{ow} \simeq 7$, and then begins decreasing again for compounds of greater hydrophobicity. Note that the relationship between log K_{ow} and log t_{ss} parallels the relationship between log K_{ow} and log K_B as shown in Figure 5. The explanations for these phenomena probably involve both influences of abiotic sorption kinetics (affecting bioavailability) and rates of transfer across the gills of organisms (affecting uptake and elimination).

113. The second step in estimating steady state from single-time-point tissue samples is to determine the half-life of the chemical. The half-life ($t_{1/2}$) is the amount of time needed for one-half of the chemical to be eliminated from the organism, and is a constant determined by the equation:

$$t_{1/2} = 0.693/k_2 \quad (17)$$

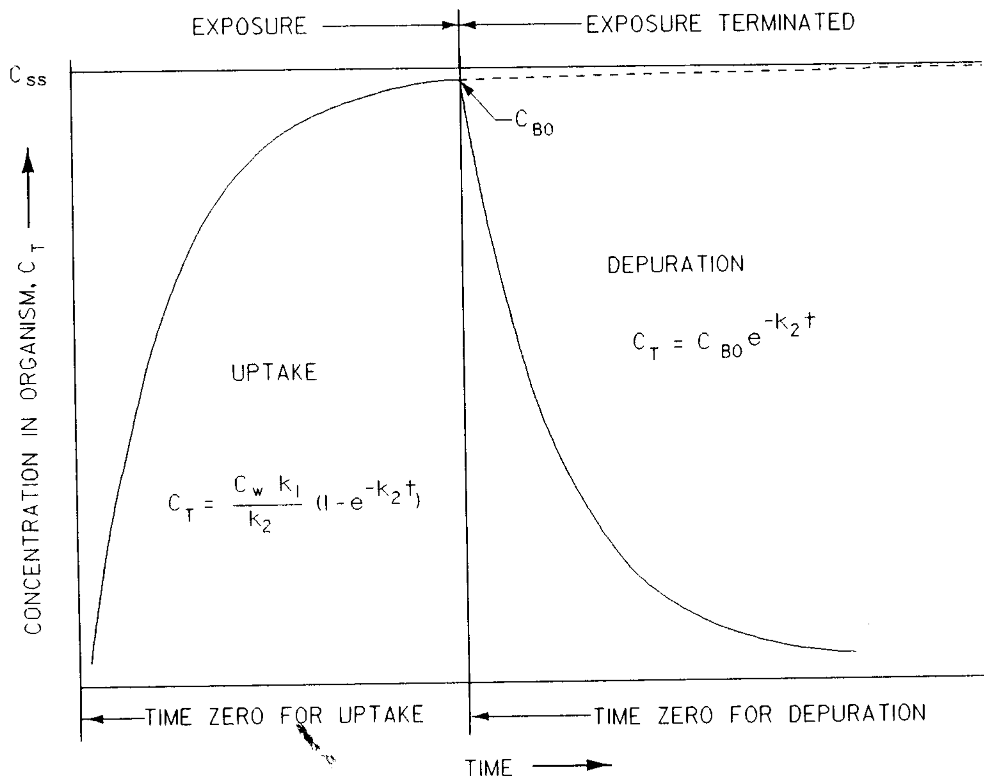


Figure 11. First-order uptake and depuration patterns (adapted with permission from Connell 1990)

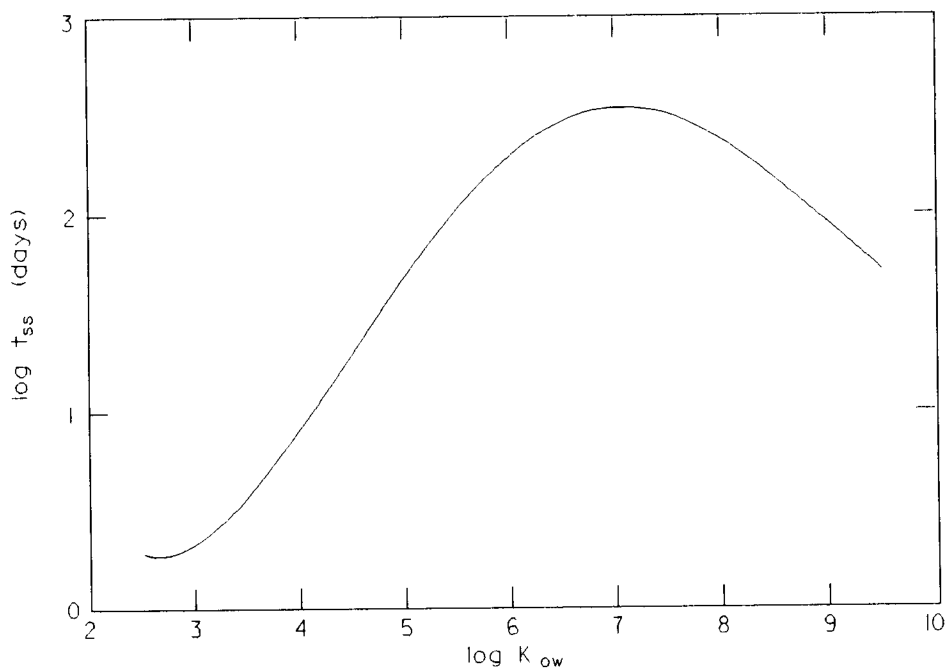


Figure 12. Relationship of log t_{ss} to log k_{ow} as described by equation for bioconcentration of chlorinated hydrocarbons and closely related compounds by fish (adapted with permission from Connell and Hawker 1988)

where k_2 is the elimination rate constant and 0.693 = -ln 0.5 (Goldstein, Aronow, and Kalman 1974). Equation 17 is obtained from the equation for time (5) to a proportion (P) of steady state:

$$t = -\ln(1 - P)/k_2 \quad (18)$$

where P for $t_{1/2}$ is one half, or 0.5. Based on Equation 18, one can calculate how many half-lives are required to reach various proportions of steady state by the following comparison:

$$[\ -\ln(1 - P)/k_2]/[\ -\ln 0.5/k_2]$$

the k_2 terms cancel, thus:

$$-\ln(1 - P)/0.693$$

For 90 percent of steady state:

$$-\ln(1 - 0.9)/0.693 = 2.303/0.693 = 3.32 \text{ half-lives}$$

For 95 percent of steady state:

$$-\ln(1 - 0.95)/0.693 = 2.996/0.693 = 4.32 \text{ half-lives}$$

For 99 percent of steady state:

$$-\ln(1 - 0.99)/0.693 = 4.605/0.693 = 6.65 \text{ half-lives}$$

And for 100 percent of steady state:

$$-\ln(1 - 1)/0.693 = \infty \quad (\ln 0 \text{ is undefined})$$

So, to determine the half-life of the contaminant of concern, one can use the t_{ss} estimated in Equation 16, and divide it by the number of half-lives required to reach t_{ss} at 99 percent of steady state:

$$t_{1/2} = t_{ss}/6.65 \quad (19)$$

(Recall that 99 percent of steady state is used because an infinite number of half-lives would be required to reach 100 percent of steady state).

114. The third step is to calculate the elimination rate constant (k_2) by rearranging Equation 17:

$$k_2 = 0.693/t_{1/2} \quad (20)$$

Insert the value of $t_{1/2}$ calculated in Equation 19.

115. The fourth step is to calculate the proportion (P) of steady state achieved at the end of the laboratory exposure (28 days for neutral organic chemicals). This can be done by rearranging Equation 18 and expressing it in exponential form:

$$P = 1 - e^{-k_2 t} \quad (21)$$

where t would normally = 28 and k_2 is as calculated in Equation 20.

116. The final step is to calculate the projected concentration at steady state C_{ss} based on the measured concentration (C_T) in the organism tissues at 28 days:

$$C_{ss} = C_T/P \quad (22)$$

where P was determined in Equation 21.

117. The following is an example to illustrate the calculations for projecting steady-state tissue concentrations in organisms exposed to contaminated sediment for 28 days. Again, DDT is the contaminant of concern, and 5.75 will be used as the value of log K_{ow} for DDT.

Step 1. Estimate time to steady state t_{ss} using log K_{ow} (Equation 16):

$$\log t_{ss} = 6.9 \times 10^{-3}(5.75)^4 - 1.85 \times 10^{-1}(5.75)^3 + 1.65(5.75)^2 - 5.34(5.75) + 5.93$$

$$\log t_{ss} = 7.54 - 35.17 + 54.55 - 30.71 + 5.93$$

$$\log t_{ss} = 2.14$$

$$t_{ss} = 138 \text{ days for DDT}$$

Step 2. Calculate half-life $t_{1/2}$ using t_{ss} (Equation 19):

$$t_{1/2} = 138 \text{ days}/6.65$$

$$t_{1/2} = 20.8 \text{ days for DDT}$$

Step 3. Calculate elimination rate constant k_2 using $t_{1/2}$ (Equation 20):

$$k_2 = 0.693/20.8 \text{ days}$$

$$k_2 = 0.0333 \text{ d}^{-1}$$

Step 4. Calculate proportion P of steady state achieved at 28 days, using k_2 (Equation 21):

$$P = 1 - e^{-(0.0333)(28)}$$

$$P = 1 - 0.39$$

$$P = 0.61$$

Step 5. Calculate projected concentration at steady state C_{ss} using proportion P of steady state and measured concentration of DDT in tissues after 28 days (Equation 22). Suppose, for example, that the average DDT concentration in organisms exposed to the contaminated sediment for 28 days was 2.0 ppm:

$$C_{ss} = 2.0/0.61$$

$$C_{ss} = 3.28 \text{ ppm}$$

Thus, based only on the 28-day tissue samples, the exposed organism would be expected to accumulate 3.28 ppm DDT at steady state.

Tier IV bioaccumulation testing

118. Bioaccumulation testing in Tier IV permits empirical determination or projection of contaminant steady-state tissue concentrations. If field-collected organisms are used, one must assume them to be at steady state with the sediments in which they are collected. In laboratory determinations, steady state is projected by means of curve fitting from time-sequenced tissue concentration data.

119. In laboratory determinations, organisms exposed to contaminated dredged material or to a reference sediment in replicate aquaria are sampled periodically over an exposure period of 28 days or more. Samples are usually taken more frequently at the beginning of the exposure period than at the end

to enable more accurate fitting of the initial, rapidly increasing portion of the uptake curve. A typical schedule for collection of tissue samples is on days 0, 2, 4, 7, 10, 18, and 28. Tier IV bioaccumulation testing could be conducted concurrently with Tier III. In this case, the time-sequenced samples would be archived and only the 28-day samples analyzed initially. If comparisons or calculations based on the 28-day samples indicate that more information is needed, then the archived samples would be analyzed.

120. Data from the time-sequenced samples are then used in a first-order, one-compartment model as described by Equations 4 through 6. The data are subjected to iterative nonlinear curve-fitting regression methods to obtain estimates of the uptake and elimination rate constants, k_1 and k_2. From the rate constants and the exposure water concentration (C_w), the projected steady-state tissue concentration C_{ss} is calculated. The data are then interpreted by comparing C_{ss} for the dredged material with C_{ss} for the reference sediment.

121. An example of a computerized nonlinear regression procedure that may be used for curve fitting in Tier IV is the SAS® NLIN procedure (SAS 1988). This procedure allows a choice of several iterative algorithms, including the Marquardt method, which is usually successful in converging to values for the rate constants, given a reasonable fit of the data to the model. Figure 13 presents an example of SAS statements using the nonlinear regression procedure (PROC NLIN) to calculate rate constants for the first-order, one-compartment bioaccumulation model, with an explanation of each statement.

Calculating bioavailability

122. As a final step in the assessment of bioaccumulation, it may be useful to determine how much of a sediment contaminant is actually bioavailable to organisms exposed to that sediment. One measure of bioavailability, p, is the proportion of the theoretical bioaccumulation potential (TBP) that is actually achieved at steady state (C_{ss}). Bioavailability is calculated as simply:

$$p = C_{ss}/TBP \tag{23}$$

Thus, this measure of bioavailability is the concentration of chemical that an organism would actually have at steady state compared to the concentration that the organism could accumulate from the sediment under ideal conditions.

```
PROC NLIN BEST=10 METHOD=MARQUARDT;                                  (1)
   PARMS K1= 0 TO 5 BY .25 K2 = .01 TO 5 BY .25;                     (2)
   VAR1= EXP(-K2*DAY);                                               (3)
   CW = 1;                                                           (4)
   MODEL CT = CW*(K1/K2)*(1-VAR1);                                   (5)
   DER.K1= CW/K2*(1-VAR1);                                           (6)
   DER.K2 = CW*(K1/K2)*(DAY*VAR1-(1-VAR1)/K2);                       (7)
```

EXPLANATION

Note: SAS statements are not numbered, nor is capitalization or indentation required. Statement numbers refer to the explanations below.

(1) Specifies the NLIN procedure with the Marquardt algorithm and the 10 best combinations of possible starting values for k_1 and k_2 (those having the lowest residual sum of squares).

(2) Parameters statement specifying a range of starting values and incremental values for k_1 and k_2.

(3) Defines a temporary variable (VAR1 = $e^{-k_2 t}$, where t = DAY)

(4) Specifies the water concentration C_w of the contaminant. If C_w is not known, a default value of 1 may be used.

(5) Specifies the first-order, one-compartment kinetic model:

$$C_T = \frac{C_w k_1}{k_2}\left[1 - e^{-k_2 t}\right]$$

where CT and DAY are values for contaminant tissue concentration and time that have been previously read into a SAS data step from data statements, a SAS data set, or an external data set.

(6) Specifies the derivative for k_1.

(7) Specifies the derivative for k_2.

Figure 13. Example of SAS statements using the nonlinear regression procedure (PROC NLIN) to calculate rate constants for the first-order, one-compartment bioaccumulation model

A p value of 1 would indicate total bioavailability, i.e., the organism has accumulated all of the chemical that it can accumulate from that sediment as the only source of exposure. Values of $p < 1$ could indicate that some of the contaminant in the sediment is not bioavailable or is metabolized. Conversely, p values > 1 may be an indication that organisms have previously or concurrently accumulated tissue residues from sources other than the sediment tested.

123. In laboratory exposures of fish and bivalves to several sediments of varying degrees of contamination, McFarland and Clarke (1986) determined p values that were generally much less than 1. Bioavailability was higher at 20° C than at 4° C, and tended to decrease with increasing degree of sediment contamination. Clarke, McFarland, and Dorkin (1988) determined p values for field-collected fish and invertebrates exposed to contaminated sediments in their natural environment. Again, the highest p values (generally much greater than 1) were for organisms from the least-contaminated environment. Organisms exposed to more highly contaminated sediments generally had p values < 1. Bioavailability for organisms collected inside a CDF ranged from about 1 to 2. The authors postulated that the CDF represented a closed aquatic ecosystem in which bioaccumulation of neutral organic chemicals was near steady state.

Example Calculations for Each Method of Bioaccumulation Assessment

124. The intent of this section is to compare potential (Tier II) and actual (Tiers III and IV) bioaccumulation of a neutral organic chemical determined using each of the four methods presented in this paper: (a) bioaccumulation potential calculated from partition coefficients, (b) TBP, (c) steady-state bioaccumulation estimated from log K_{ow} and single-time-point tissue samples, and (d) steady-state bioaccumulation projected from time-sequence-collected tissue samples. Additionally, calculation of bioavailability will be presented. Calculations are for the PCB congener No. 101 (2,2',4,5,5'-pentachlorobiphenyl) using actual data from laboratory exposures of fish (fathead minnows) and clams (*Corbicula*) to a contaminated sediment from the Hudson River, NY. PCB uptake and lipid data for the animals are given in Table 5. The reader should note that this experiment does not follow all of the USEPA (1990) guidelines for bioaccumulation testing, and is meant only to illustrate the bioaccumulation calculations, not the laboratory

Table 5

PCB Bioaccumulation and Lipid Data for Clams and Fish

Exposed to Contaminated Hudson River Sediment

Day	PCB Congener 101, ppm		Lipid, percent	
	Clams	Fish	Clams	Fish
1	0.04	0.03	2.29	3.97
1	0.05	0.03	2.41	5.87
1	0.06	0.03	2.01	5.47
2	0.08	0.07	2.44	4.65
2	0.08	0.09	1.64	5.03
2	0.10	0.07	5.85	5.08
4	0.11	0.11	1.77	3.67
4	0.10	0.14	1.64	3.94
4	0.10	0.14	1.79	4.01
7	0.20	0.16	1.65	3.65
7	0.26	0.22	1.77	4.23
7	0.19	0.21	1.23	4.20
11	0.32	0.52	1.80	6.00
11	0.25	0.38	2.10	4.80
11	0.40	0.47	2.00	3.00
18	0.29	0.65	2.60	6.10
18	0.25	0.35	2.50	2.40
18	0.31	0.43	2.30	4.10

procedures or the decision-making (dredged material-to-reference) comparisons specified in the USEPA (1990) guidance.

125. The following partition coefficients will be used for PCB 101:

$\log K_{ow} = 6.50$ (Woodburn, Doucette, and Andren 1984)

$\log K_{oc} = 5.65$ (Baker, Capel, and Eisenreich 1986)

$\log K_B = 4.66$ for several species of fish, including fathead minnows, in a flowing water ecosystem (Kenaga and Goring 1980)

Analysis of the exposure sediment and water yielded:

$C_s = 0.17$ ppm PCB 101

TOC = 5.85 percent

$C_w = 0.00001$ ppm PCB 101

(1) Estimating bioaccumulation
potential from partitioning coefficients

126. Using this estimation method, it is necessary to know the sediment concentration of the contaminant (C_s = 0.17 ppm) and total organic carbon content (TOC = 5.85 percent), and the partition coefficients for PCB 101. If all three partition coefficients (K_{ow}, K_{oc}, and K_B) are known, then:

$$C_s^{oc} = C_s/TOC = 0.17/0.585 = 2.91 \text{ ppm} \quad (8)$$

$$C_w = C_s^{oc}/K_{oc} = 2.91/\text{antilog } 5.65 = 0.0000065 \text{ ppm} \quad (9)$$

$$C_B = K_B \times C_w = \text{antilog } 4.66 \times 0.0000065 = 0.30 \text{ ppm} \quad (10)$$

Thus, one would expect an organism exposed to this sediment to bioaccumulate 0.30 ppm PCB 101 at steady state.

127. If, however, only K_{ow} is known, then one must first estimate K_{oc} and K_B from K_{ow}:

$$\log K_{oc} = 0.989 \log K_{ow} - 0.346 \quad (11)$$
$$= (0.989 \times 6.50) - 0.346 = 6.08$$

$$\log K_B^{lipid} = 0.980 \log K_{ow} - 0.063 \quad (12)$$
$$= (0.980 \times 6.50) - 0.063 = 6.31$$

Then

$$C_s^{oc} = 2.91 \text{ ppm} \quad (8)$$

$$C_w = 2.91/\text{antilog } 6.08 = 0.0000024 \text{ ppm} \quad (9)$$

$$C_B^{lipid} = \text{antilog } 6.31 \times 0.0000024 = 4.90 \text{ ppm} \quad (10)$$

To convert C_B^{lipid} to whole-body bioaccumulation, one could use the mean lipid content of the clams and fish, calculated from Table 4:

$$\text{clams:}\quad C_B \text{ (whole body)} = C_B^{lipid} \times fL$$
$$= 4.90 \times 0.0221 = 0.11 \text{ ppm} \qquad (13)$$
$$\text{fish:}\quad C_B \text{ (whole body)} = 4.90 \times 0.0445 = 0.22 \text{ ppm}$$

Thus, using estimated K_{oc} and K_B, one would expect the clams to accumulate 0.11 ppm and the fish to accumulate 0.22 ppm PCB 101 at steady state.

(2) TBP

128. To estimate bioaccumulation potential using the TBP method, one need know only the sediment contaminant concentration (C_s) and TOC, the preference factor (pf), and the lipid content (fL) of the organisms of interest. For these calculations, pf = 4 will be used, as specified in USEPA (1990):

$$TBP = pf \times (C_s/TOC) \times fL \qquad (15)$$

For clams:

$$TBP = 4 \times (0.17/0.0585) \times 0.0221 = 0.26 \text{ ppm}$$

For fish:

$$TBP = 4 \times (0.17/0.0585) \times 0.0445 = 0.52 \text{ ppm}$$

Thus, using the TBP method, one would expect the clams to accumulate 0.26 ppm and the fish to accumulate 0.52 ppm PCB 101 at steady state.

(3) Steady-state bioaccumulation estimated from log K_{ow} and single-time-point tissue samples

129. Using this method from Tier III bioaccumulation testing requires only the PCB 101 concentrations in clams and fish determined at the end of the laboratory exposure period (day 18 in Table 5). First, estimate the time to steady state using Connell and Hawker's polynomial equation:

$$\begin{aligned} \log t_{ss} &= 6.9 \times 10^{-3}(\log K_{ow})^4 - 1.85 \times 10^{-1}(\log K_{ow})^3 \\ &\quad + 1.65(\log K_{ow})^2 - 5.34(\log K_{ow}) + 5.93 \\ &= 6.9 \times 10^{-3}(6.50)^4 - 1.85 \times 10^{-1}(6.50)^3 \\ &\quad + 1.65(6.50)^2 - 5.34(6.50) + 5.93 \qquad (16) \\ &= 12.32 - 50.81 + 69.71 - 34.71 + 5.93 \\ &= 2.44 \\ t_{ss} &= 275 \text{ days for PCB 101} \end{aligned}$$

Now, calculate the half-life:

$$t_{1/2} = t_{ss}/6.65$$
$$= 275 \text{ days}/6.65 = 41.35 \text{ days for PCB 101} \quad (19)$$

Next, calculate the elimination rate constant:

$$k_2 = 0.693/t_{1/2}$$
$$= 0.693/41.35 = 0.0168 \text{ d}^{-1} \quad (20)$$

Now, determine the proportion of steady state achieved at 18 days:

$$P = 1 - e^{-k_2 t}$$
$$= 1 - e^{-(0.0168)(18)} \quad (21)$$
$$= 1 - 0.74 = 0.26$$

Finally, calculate the projected concentration at steady state based on average measured concentrations in tissues after 18 day's exposure:

$$C_{ss} = C_T/P \quad (22)$$

For clams:

$$C_{ss} = 0.28/0.26 = 1.08 \text{ ppm PCB 101}$$

For fish:

$$C_{ss} = 0.48/0.26 = 1.85 \text{ ppm PCB 101}$$

(4) Steady-state bioaccumulation projected from time-sequence-collected tissue samples

130. The full bioaccumulation data from Table 5 can be used to estimate rate constants, fit uptake curves, and project steady-state tissue concentrations for PCB 101. This has been done using SAS on an IBM-compatible personal computer, with the SAS statements illustrated in Figure 13. The parameters statement (statement 2) was modified to provide higher starting values for K_1:

```
parms k1 = 100 to 10000 by 200 k2 = .01 to 5 by .25;
```

and statement 4 used the actual water concentration (C_w = 0.00001 ppm) for PCB 101 in this experiment. The SAS NLIN program generated the following values and 95-percent confidence intervals for the rate constants:

clams: $K_1 = 4838.9$ (3100.3, 6577.5)
$K_2 = 0.14286$ (0.06023, 0.22549)

fish: $k_1 = 4296.4$ (2386.9, 6205.9)
$k_2 = 0.05086$ (-0.01999, 0.12172)

Now, calculate steady state tissue concentrations for PCB 101:

$$C_{ss} = (k_1 C_w)/k_2 \quad (6)$$

For clams:

$$C_{ss} = 4838.9(0.00001)/0.14286 = 0.34 \text{ ppm PCB 101}$$

For fish:

$$C_{ss} = 4296.4(0.00001)/0.05086 = 0.84 \text{ ppm PCB 101}$$

The proportion of steady state achieved at day 18:

clams: $P = 1 - e^{-(0.14286)(18)} = 0.92$
fish: $P = 1 - e^{-(0.05086)(18)} = 0.60$

One can also determine the half-life of PCB 101 and the time to 99 percent of steady state by rearranging Equations 20 and 19:

clams: $t_{1/2} = 0.693/k_2 = 0.693/0.14286 = 4.85$ days
$t_{ss} = t_{1/2} \times 6.65 = 4.85 \times 6.65 = 32.25$ days

fish: $t_{1/2} = 0.693/0.05086 = 13.63$ days
$t_{ss} = 13.63 \times 6.65 = 90.64$ days

The actual data and the fitted regression curves are plotted in Figure 14. The SAS program statements used to generate this plot and print it on a Hewlett-Packard Laserjet Plus printer are given in Appendix C.

Calculation of bioavailability

131. Having determined tissue concentrations at steady state, one can now calculate bioavailability:

$$p = C_{ss}/TBP \qquad (23)$$

Using the C_{ss} values determined in Method (3):

clams: $p = 1.08/0.26 = 4.15$

fish: $p = 1.85/0.52 = 3.56$

Using the C_{ss} values determined in Method (4):

clams: $p = 0.34/0.26 = 1.31$

fish: $p = 0.84/0.52 = 1.62$

These p values suggest that using single-time-point tissue concentrations to estimate steady state for PCB 101 (Method 3) led to an overestimation of C_{ss}. Lower values for C_{ss} were obtained using the time-sequenced bioaccumulation data (Method 4), resulting in p values close to the expected value of unity for a system in which all of the sediment contaminant is bioavailable.

132. Table 6 summarizes values for all of the parameters used or calculated in the four methods demonstrated above for PCB 101. Parameter values indicated by dashes are not needed or calculated for a particular method. In this particular example, all of the methods result in estimated or projected steady state tissue concentrations of PCB 101 that are quite similar to each other. Only Method 3 projects $C_{ss} > 1$ ppm. Recall that Methods 1 and 2 are theoretical "paper exercises" that require knowledge of sediment contaminant concentration and TOC. Method 1 also requires knowledge of contaminant partition coefficients. Methods 3 and 4 are empirical determinations that require actual exposures of organisms to the contaminated sediment, but do not require knowledge of the sediment chemistry. Method 4 calculations require the concentration of contaminant in the exposure water, but if this is not known, a default value of 1 can be substituted. The calculated uptake rate constant

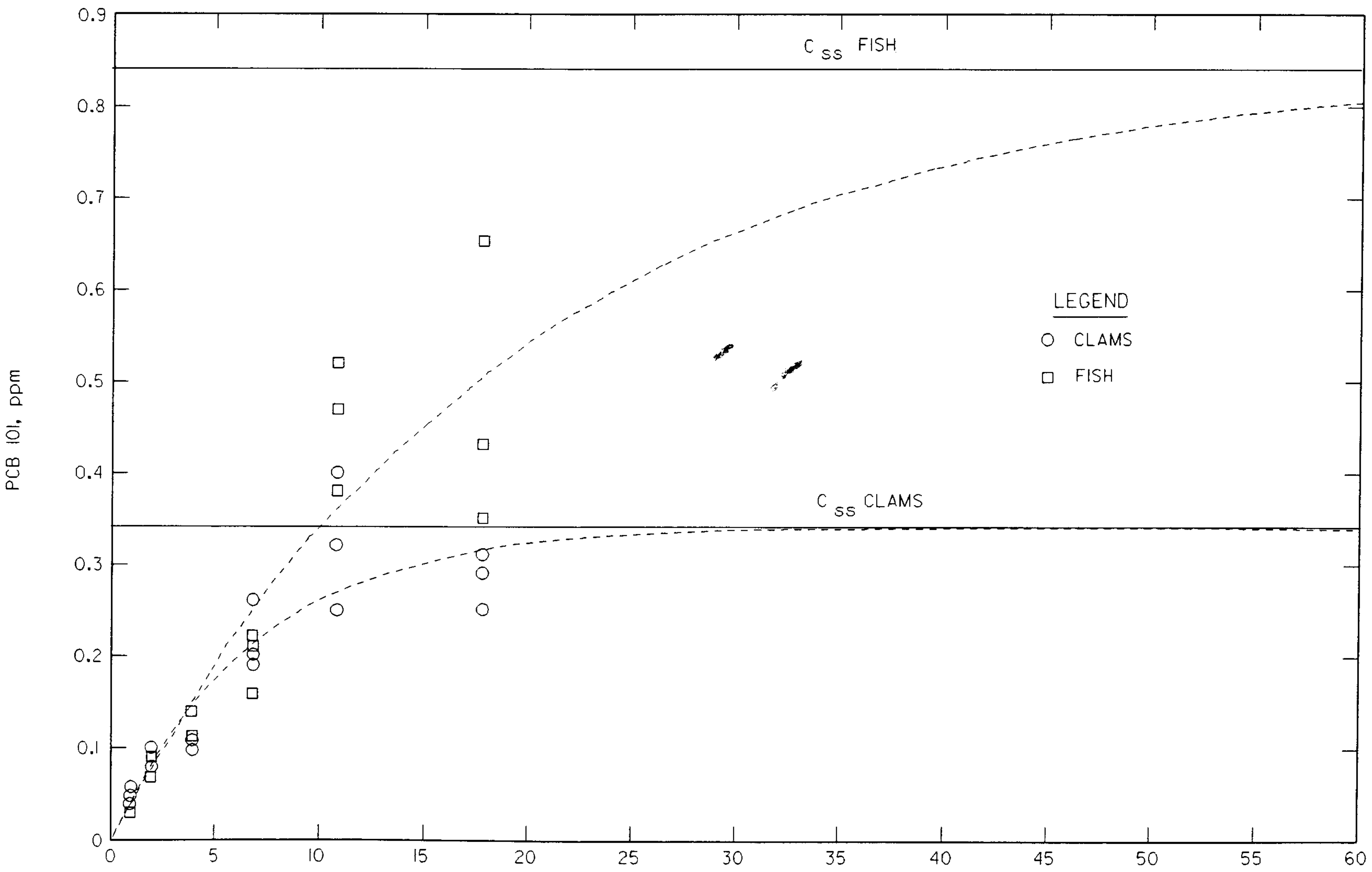


Figure 14. Bioaccumulation of PCB 101 from Hudson River sediment by clams and fish, with projected steady-state tissue concentrations, etc.

(k_1) will then be meaningless, but the elimination rate constant (k_2) and other calculated parameters will still be accurate.

133. In summary, Methods 1 and 2 are appropriate for theoretical bioaccumulation estimations in Tier II bioaccumulation evaluations, as an inexpensive screening tool to determine whether further bioaccumulation testing is required. Method 2 (TBP) in particular is quick, simple, and requires knowledge only of the sediment contaminant concentration and TOC, and organism lipid content. Method 3 can be used to project contaminant steady-state tissue concentrations from Tier III bioaccumulation testing. If more accurate steady-state projections are needed, then Method 4 can be conducted with Tier IV bioaccumulation testing. This requires the added expense of analyzing more tissue samples, along with more involved, computer-assisted data analysis, for a truer picture of what maximum contaminant bioaccumulation from a given sediment is likely to be.

Table 6

Comparison of Parameter Values From Four Methods for Determining Bioaccumulation of PCB 101*

Parameter	Method for Determining Bioaccumulation				
	1**	1†	2	3	4
log K_{ow}	6.50††	6.50	--	6.50	--
log K_{oc}	5.65	*6.08*	--	--	--
log K_B	4.66	*6.31*	--	--	--
C_s, ppm	0.17	0.17	0.17	--	--
TOC	0.0585	0.0585	0.0585	--	--
C_s^{oc}, ppm	*2.91*	*2.91*	--	--	--
C_w, ppm	*0.0000065*	*0.0000024*	--	--	0.00001
C_{ss}, ppm	*0.30*‡	--	--	--	--
clams	--	*0.11*‡	*0.26*‡‡	1.08	0.34
fish	--	*0.22*‡	*0.52*‡‡	1.85	0.84
fL					
clams	--	0.0221	0.0221	--	--
fish	--	0.0445	0.0445	--	--
t_{ss}, days	--	--	--	*275*	--
clams	--	--	--	--	32.25
fish	--	--	--	--	90.64
$t_{1/2}$, days	--	--	--	*41.35*	--
clams	--	--	--	--	4.85
fish	--	--	--	--	13.63
k_1					
clams	--	--	--	--	4838.9
fish	--	--	--	--	4296.4
k_2	--	--	--	*0.0168*	--
clams	--	--	--	--	0.14286
fish	--	--	--	--	0.05086
P of C_{ss} at 18 days	--	--	--	0.26	--
clams	--	--	--	--	0.92
fish	--	--	--	--	0.60
p (bioavailability)					
clams	--	--	--	4.15	1.31
fish	--	--	--	3.56	1.62

* (1) Estimating bioaccumulation potential from partition coefficients, (2) TBP, (3) Projecting steady-state bioaccumulation from log K_{ow} and single-time-point tissue samples, and (4) Projecting steady-state bioaccumulation from time-sequenced tissue samples.

** Log K_{ow}, log K_{oc}, and log K_B all known *a priori*.

† Only log K_{ow} known *a priori*; log K_{oc} and log K_B estimated from log K_{ow}.

†† Parameter values in regular type are known *a priori* or determined from the laboratory bioaccumulation data for PCB 101; parameter values in italics are estimated from theoretical relationships.

‡ Using Method 1, $C_{ss} = C_B$.

‡‡ Using Method 2, C_{ss} = TBP .

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APPENDIX A

NOTATION

Å	Angstrom
AF	Accumulation factor
APF	Apparent preference factor
C	Concentration
C_a	Concentration of chemical in air
Ca^{++}	Calcium ion
C_B	Concentration of chemical in biota
C_B^{lipid}	Concentration of chemical in lipids of biota
CCL_4	Carbon tetrachloride
CDF	Confined disposal facility
C_{doc}	Concentration of chemical in dissolved organic carbon
Cd^{++}	Cadmium ion
$CHCl_3$	Chloroform
C_i	Concentration of chemical in pure solute
C^{lipid}	Concentration of chemical in lipid
C_{oct}	Concentration of chemical in octanol
Cr	Chromium
Cr^{+2}	Chromous ion
Cr^{+3}	Chromic ion
C_s	Concentration of chemical in sediment
C_s^{oc}	Concentration of chemical in soil or sediment organic carbon
C_{sp}	Concentration of chemical in suspended particulates
C_{sp}^{oc}	Concentration of chemical in suspended particulate organic carbon
C_{ss}	Concentration of chemical in organism tissues at steady state
C_T	Concentration of chemical in tissues of an organism
C_w	Concentration of chemical in water
d	Days
DDD	Dichloro-diphenyl-dichloroethane
DDE	Dichloro-diphenyl-dichloroethylene
DDT	Dichloro-diphenyl-trichloroethane
DOC	Dissolved organic carbon
e	Exponential
Eh	Oxidation-reduction potential

f	Fugacity
FDA	Food and Drug Administration
fL	Fraction of lipid
f_o^B	Fugacity of benzene in octanol
f_w^B	Fugacity of benzene in water
f_w^o	Fugacity of octanol in water
f_o^w	Fugacity of water in octanol
g/ml	Grams per milliliter
h	Hours
H	Air:water partition coefficient (Henry's Law constant)
H^+	Proton
H_3O^+	Hydronium ion
k_1	Uptake rate constant
k_2	Elimination rate constant
K_B	Organism:water partition coefficient (= bioconcentration factor)
K_B^{lipid}	Organism lipid:water partition coefficient
K_d	Soil or sediment:water partition coefficient
K_{oc}	Soil or sediment organic carbon:water partition coefficient
K_{ow}	Octanol:water partition coefficient
K_{sp}	Suspended particulate:water partition coefficient
ln	Natural logarithm ($base_e$)
log	Logarithm ($base_{10}$)
MFO	Mixed-function oxidase
Mg^{++}	Magnesium ion
mg/kg	Milligrams per kilogram (ppm)
mv	Millivolts
mw	Molecular weight
ng/g	Nanograms per gram (ppb)
ng/ℓ	Nanograms per liter (pptr)
OH^-	Hydroxyl ion
p	Bioavailability
P	Proportion
PAH	Polynuclear aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCB 101	2,2',4,5,5'-pentachlorobiphenyl
pf	Preference factor
PF	Partitioning factor

pH	Acidity/basicity
pKa	Acid dissociation constant
ppb	Parts per billion
ppm	Parts per million
pptr	Parts per trillion
r^2	Coefficient of determination
S	Pure chemical:water partition coefficient (aqueous solubility)
t	Time
TBP	Thermodynamic (theoretical) bioaccumulation potential
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCDF	2,3,7,8-tetrachlorodibenzofuran
TOC	Total organic carbon
t_{ss}	Time to 99 percent of steady state
$t_{1/2}$	Half-life
USEPA	United States Environmental Protection Agency
Z	Fugacity capacity constant
μ	Microns (or μm micrometers)
μg/g	Micrograms per gram (ppm)
μg/ℓ	Micrograms per liter (ppb)
°C	Degrees Celcius
∞	Infinity

APPENDIX B

SUMMARY OF EQUATIONS

1. $C = Zf$
2. $pKa - pH = \log([nonionized]/[ionized])$ (acids)
3. $pKa - pH = \log([ionized]/nonionized])$ (bases)
4. $dC_T/dt = k_1C_w - k_2C_T$
5. $C_T = ((k_1C_w)/k_2)(1 - e^{-k_2t})$
6. $C_{ss} = (k_1C_w)/k_2$
7. $K_B = k_1/k_2 = C_{ss}/C_w$
8. $C_s^{oc} = C_s/TOC$
9. $C_w = C_s^{oc}/K_{oc}$
10. $C_B = K_B \times C_w$
11. $\log K_{oc} = 0.989 \log K_{ow} - 0.346$
12. $\log K_B^{lipid} = 0.980 \log K_{ow} - 0.063$
13. C_B (whole body) $= C_B^{lipid} \times fL$
14. $pf = C_B^{lipid}/C_s^{oc}$
15. $TBP = pf \times (C_s/TOC) \times fL$
16. $\log t_{ss} = 6.9 \times 10^{-3}(\log K_{ow})^4 - 1.85 \times 10^{-1}(\log K_{ow})^3 + 1.65(\log K_{ow})^2 - 5.34(\log K_{ow}) + 5.93$
17. $t_{1/2} = 0.693/k_2$
18. $t = -\ln(1 - P)/k_2$
19. $t_{1/2} = t_{ss}/6.65$
20. $k_2 = 0.693/t_{1/2}$
21. $P = 1 - e^{-k_2t}$
22. $C_{ss} = C_T/P$
23. $p = C_{ss}/TBP$

APPENDIX C

SAS PROGRAM STATEMENTS* FOR PLOTTING BIOACCUMULATION DATA AND FITTED REGRESSION CURVES

```
LIBNAME Q 'C:\SAS';
DATA GEN1;
 K1= 4838.897275;
 K2= 0.142858;
 CW= .00001;
 CI= 0;
 DO DAY = 0 TO 60 BY .1;
  IF DAY = 0 THEN LINE1 = CI;
  ELSE DO;
   TEMP = EXP(-K2*DAY);
   TEMP2 = (CW*K1/K2)*(1-TEMP);
   LINE1 = CI*TEMP + TEMP2;
   OUTPUT;
   END;
  END;
 KEEP DAY LINE1;
DATA GEN2;
 K1= 4296.40011;
 K2= 0.050864;
 CW= .00001;
 CI= 0;
 DO DAY = 0 TO 60 BY .1;
  IF DAY = 0 THEN LINE2 = CI;
  ELSE DO;
   TEMP = EXP(-K2*DAY);
   TEMP2 = (CW*K1/K2)*(1-TEMP);
   LINE2 = (CI*TEMP + TEMP2;
   OUTPUT;
   END;
  END;
 KEEP DAY LINE2;
DATA A;
 SET Q.PCB101 GEN1 GEN2;
 GOPTIONS DEVICE=HPLJ5P2 GACCESS='SASGASTD>LPT2:' ROTATE=LANDSCAPE;
 TITLE ' ';
 FOOTNOTE '* = CLAMS    [] = FISH';
PROC GPLOT;
 AXIS1 LABEL=(A=90 'PCB 101, ppm')
       ORDER= 0 TO .9 BY .1;
 PLOT PCBCLAM*DAY LINE1*DAY PCBFISH*DAY LINE2*DAY / OVERLAY
   HAXIS= 0 TO 60 BY 5 VAXIS=AXIS1 VREF = .34 .84;
 SYMBOL1 V=STAR;
 SYMBOL2 V=NONE L=1 I=JOIN;
 SYMBOL3 V=SQUARE;
 SYMBOL4 V=NONE L=2 I=JOIN;
```

* SAS requires a semicolon at the end of each statement but does not require capitalization or indentation.