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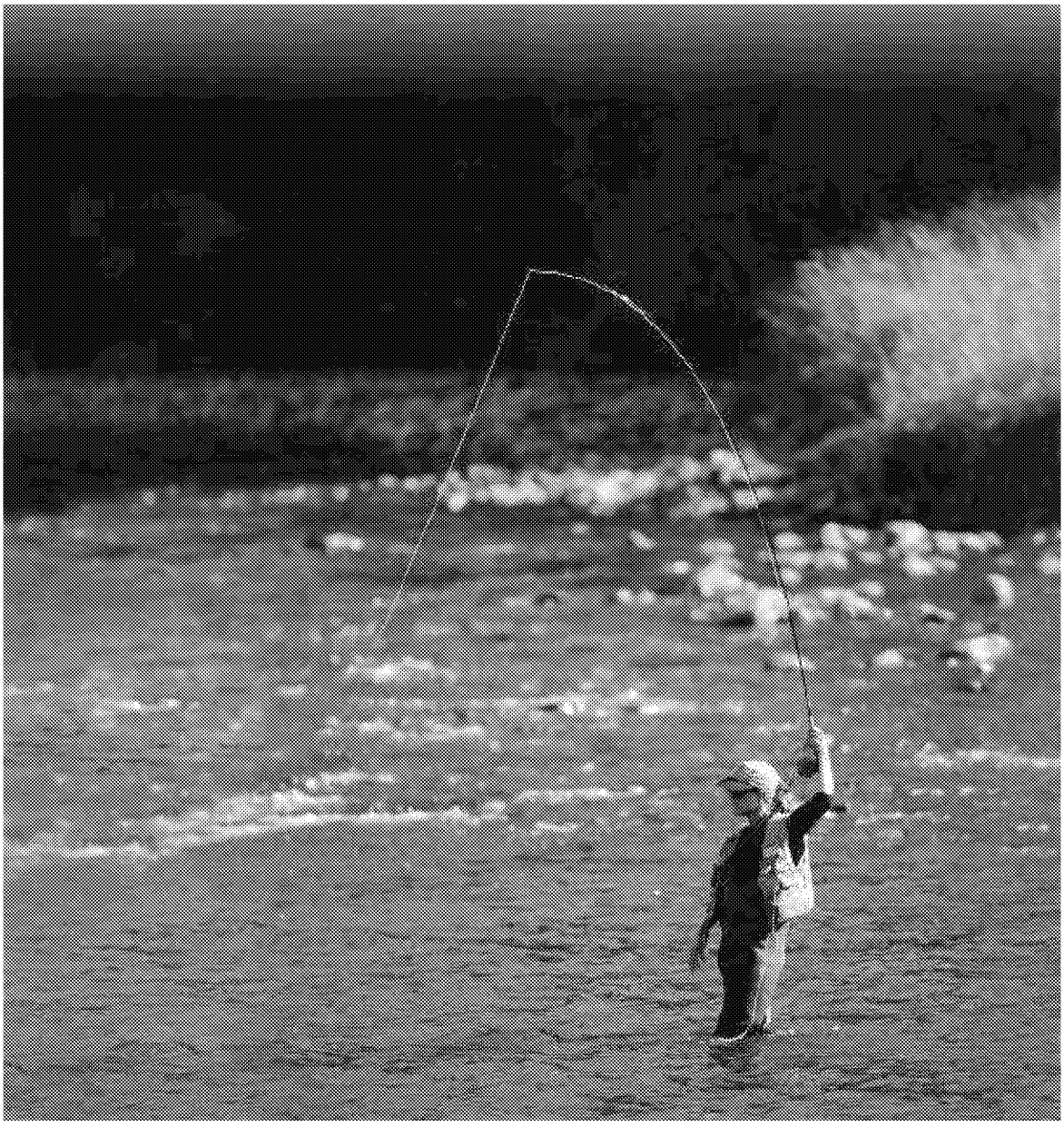
Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)

Technical Support Document
Volume 3: Development of Site-Specific Bioaccumulation Factors



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Technical Support Document Volume 3: Development of Site-Specific Bioaccumulation Factors

Office of Science and Technology
Office of Water
U.S. Environmental Protection Agency
Washington, DC 20460

NOTICE

The policies and procedures set forth in this document are intended solely to describe EPA methods and guidance for developing or revising ambient water quality criteria to protect human health, pursuant to Section 304(a) of the Clean Water Act, and to serve as guidance to States and authorized Tribes for developing their own water quality criteria. This guidance does not substitute for the Clean Water Act or EPA's regulations, nor is it a regulation itself. Thus, it does not impose legally binding requirements on EPA, States, Tribes, or the regulated community, and may not apply to a particular situation depending on the circumstances.

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FOREWORD

This *Technical Support Document Volume* 3: *Development of Site-Specific Bioaccumulation Factors* (Site-Specific TSD) provides technical details on how state and tribal water quality staff scientists or risk assessors ("investigators"), who are responsible for deriving state or tribal water quality standards, may develop site-specific bioaccumulation factors (BAFs) for use in deriving ambient water quality criteria for protecting human health. Guidance on different approaches that investigators can take and the factors that should be considered when selecting an approach for a given situation is provided. This information allows states and tribes to derive BAFs that are more representative of the bioaccumulation potential at a given location.

The Site-Specific TSD was developed as a supplemental document to the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (Human Health Methodology) that updated and revised the existing 1980 Guidelines and Methodology. The Human Health Methodology includes guidance on chemical risk assessment, exposure and bioaccumulation.

As part of the Human Health Methodology, EPA developed detailed procedures and guidelines for estimating bioaccumulation factor (BAF) values for use in deriving or revising ambient water quality criteria. The *Technical Support Document Volume* 2: *Development of National Bioaccumulation Factors* (EPA-822-R-03-030) (National TSD) discusses the technical basis for developing BAFs, the underlying assumptions and uncertainties inherent to the approach, and applying the bioaccumulation component of the Human Health Methodology. The National and Site- Specific TSDs should be used in conjunction with the 2000 Human Health Methodology to develop BAFs for use in calculating ambient water quality criteria.

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TABLE OF CONTENTS

1. INTRODUCTION 1-1
1.1 BACKGROUND 1-3
1.2 SCOPE OF DOCUMENT 1-5
1.3 SITE-SPECIFIC BIOACCUMULATION FACTORS (SS BAFs) 1-7
1.4 GLOSSARY 1-9
REFERENCES 1-13

2. HOW TO SELECT AN APPROACH FOR DERIVING SITE-SPECIFIC BAFS 2-1
2.1 BAF DERIVATION PROCEDURES FOR INORGANIC AND ORGANOMETALLIC CHEMICALS 2-4
2.2 BAF DERIVATION PROCEDURES FOR IONIC ORGANIC CHEMICALS 2-5
2.3 BAF DERIVATION ASSUMPTIONS 2-6
2.4 WHAT IS THE DEFINITION OF A SITE? 2-7
2.5 WHAT ARE THE METHODS FOR DERIVING SITE-SPECIFIC BAFS? 2-10
2.5.1 Site-specific Field-Measured BAFs 2-11
2.5.2 Site-specific BAFs Predicted from Measured Biota Sediment Accumulation Factors (BSAFs) 2-11
2.5.3 Site-specific BAFs Predicted from Extrapolated BSAFs or BEFs Measured at a Reference Site 2-12
2.5.4 Site-specific BAFs Predicted from Laboratory Measured BCFs Combined with a Food Chain Multipliers 2-12
2.5.5 Site-specific BAFs Predicted from *N*-Octanol-Water Partition Coefficient (K_{ow}) Combined with a Food Chain Multipliers 2-13
2.5.6 Site-specific BAFs Recalculated from National or Baseline BAFs 2-14
2.5.7 Advantages and Limitations of Site-specific BAF Approaches 2-14
2.5.8 Weight-of-Evidence Approach to Selecting a Site-specific BAF 2-18
REFERENCES 2-20

3. MEASURING SITE-SPECIFIC BIOACCUMULATION FACTORS 3-1
3.1 KEY STUDY DESIGN QUESTIONS FOR DETERMINING SITE-SPECIFIC BIOACCUMULATION FACTORS 3-11
3.2 HOW TO DESIGN A SAMPLING PLAN TO MEASURE BAFS 3-17
3.2.1 Determining the Number of Samples to Collect 3-19
3.2.1.1 Bootstrap BAF Resampling 3-20
3.2.1.2 Monte Carlo BAF Analysis 3-21
3.2.2 Modeling Simulation of BAF Sampling Designs 3-22
3.2.2.1 Using Model Simulations to Develop Field-Sampling Designs 3-23
3.2.3 How Can These Methods be used to Help Design a BAF Sampling Plan? 3-26

ED_006490_00009970-00009

TABLE OF CONTENTS (CONTINUED)

3.3 MEASURING CHEMICAL CONCENTRATIONS IN BIOTA 3-28
3.3.1 Target Analytes and Analytical Methods 3-30
3.3.2 Target Species and Size Class Selection 3-31
3.3.3 Sampling Locations 3-35
3.3.4 Sampling Times 3-40
3.3.5 Sample Type 3-41
3.3.6 Replicate Samples 3-46
3.3.7 Sample Collection Methods 3-47
3.4 MEASURING CHEMICAL CONCENTRATIONS IN WATER 3-47
3.4.1 Target Analytes and Analytical Methods 3-48
3.4.2 Phase Separation 3-49
3.4.3 Sampling Locations 3-50
3.4.4 Sampling Times 3-51
3.4.5 Sample Type 3-52
3.4.6 Replicate Samples 3-52
3.4.7 Sample Collection Methods 3-53
REFERENCES 3-55

Appendix 3A: Determining the Number of Samples to Collect for a BAF Measurement: Bootstrap Analysis 3-65
Appendix 3B: PCB Congener Concentrations Measured by 79 Green Bay Mass Balance Study 3-79
Appendix 3C: Determining the Number of Samples to Collect for a BAF Measurement: Monte Carlo Analysis 3-83
Appendix 3D: Modeling Simulation of BAF Sampling Designs 3-91

4. MEASURING BIOTA-SEDIMENT ACCUMULATION FACTORS TO PREDICT SITE-SPECIFIC BIOACCUMULATION FACTORS 4-1
4.1 DESCRIPTION OF METHOD 2 4-2
4.2 KEY STUDY DESIGN QUESTIONS FOR DETERMINING BIOTA-SEDIMENT ACCUMULATION FACTORS 4-11
4.3 HOW CAN THE SEDIMENT/WATER COLUMN CHEMICAL CONCENTRATION QUOTIENT (II_{SOCW}) BE DETERMINED? 4-18
4.3.1 Measuring Site-specific Reference Chemical Concentrations in Water and Sediment 4-20
4.3.2 Estimating socw / K_{ow} ~ f_{OC}/f_{SOC} by Assuming Steady State 4-22
4.3.3 Using Transport and Fate Models to Determine the Fugacity Gradient Ratio 4-23
4.4 HOW TO DESIGN A SAMPLING PLAN TO MEASURE BSAFs 4-27
4.4.1 How Can Modeling Simulations Guide the Sampling Design Process? 4-27
4.4.2 Using Model Simulations to Develop Field-Sampling Designs 4-29

ED_006490_00009970-00010

TABLE OF CONTENTS (CONTINUED)

4.4.3 Using Monte Carlo Simulation to Determine the Number of Samples to Collect and Analyze 4-31
4.4.4 How Can These Methods be Used to Help Design a Sampling Plan? 4-33
4.5 MEASURING CHEMICAL CONCENTRATIONS IN SEDIMENT 4-35
4.5.1 Target Analytes and Analytical Methods 4-36
4.5.2 Sampling Locations and Depths 4-37
4.5.3 Sample Type and Collection Method 4-42
4.5.4 Replicate and Composite Samples 4-43
4.6 SCIENTIFIC ISSUES ASSOCIATED WITH METHOD 2 AND THE USE OF BSAFS TO PREDICT CHEMICAL BIOACCUMULATION 4-45
4.6.1 Evaluation of Method 2 Predictions of Site-specific BAFs 4-45
4.6.2 Is Chemical Equilibrium Assumed in the Calculation of a BSAF? 4-50
4.6.3 Review of Existing Data for $_{SOCW}$ 4-51
4.6.4 How does II_{SOCW} Reflect Steady State Conditions at a Site? 4-60
4.6.5 Assumptions and Limitations Associated with Method 2 Predictions 4-61
4.6.6 How Reliable are Method 2 Predictions if the Sediment Organic Carbon Equilibrium Partitioning Assumption is in Error? 4-63
REFERENCES 4-66

Appendix 4A - Modeling Simulation of BSAF Sampling Designs 4-73
Appendix 4B - Determining the Number of Samples to Collect for a BSAF Measurement: Monte Carlo Analysis 4-77
Appendix 4C - Green Bay Mass Balance PCB Congener Concentrations: Organic Carbon-Normalized Surficial (0-1 cm) Sediment 4-95

5. ESTIMATING SITE-SPECIFIC BAFs BY EXTRAPOLATION, PREDICTION OR RECALCULATION 5-1
5.1 ESTIMATING SITE-SPECIFIC BAFS BY EXTRAPOLATING BSAFS OR BEFS (METHODS 3A AND 3B) 5-3
5.1.1 Estimating Site-specific BAFs by extrapolating BSAFs (Method 3a) 5-12
5.1.2 Estimating Site-specific BAFs by extrapolating BEFs (Method 3b) 5-23
5.1.3 How Can a Reference Site be Chosen? 5-33
5.2 PREDICTING SITE-SPECIFIC BAFS USING BCFS AND FOOD CHAIN MULTIPLIERS (FCMs) 5-35
5.2.1 Predicting Site-specific Baseline BAFs using Laboratory-Measured BCFs and FCMs (Method 4a) 5-36
5.2.1.1 Validation of Method 4a 5-42
5.2.2 Determining Site-specific FCMs 5-44
5.2.2.1 Measuring Site-specific FCMs 5-46
5.2.2.2 Predicting FCMs Using a Food Chain Model 5-49
5.2.2.3 Site-specific Adjustment of Food Chain Model Parameters 5-57
5.2.2.4 Selection of a Food Web Structure 5-58
5.2.2.5 Alternative Food Chain Models 5-59

ED_006490_00009970-00011

TABLE OF CONTENTS (CONTINUED)

5.2.3 Predicting Site-specific Baseline BAFs using K_{OW} and Food Chain Multipliers (Method 4b) 5-59
5.2.3.1 Validation of Method 4b 5-65
5.2.3.2 Selection of Appropriate K_{OWS} for Partitioning (Bioavailability) Predictions 5-69
5.3 Recalculating Site-specific BAFs from Baseline or National BAFs 5-70
5.3.1 Assumptions and Limitations 5-72
5.3.2 Validation of Method 5 5-76
5.3.3 How Can the Lipid Contents of Aquatic Organisms be Determined? 5-81
5.3.3.1 Assessing Site-specific Fish Consumption 5-82
5.3.3.2 Measuring Lipid in Fish 5-82
5.3.3.3 Determining Site-specific Fish Lipid Using a Literature or Database Search 5-84
5.3.3.4 How Should Lipid Data be Evaluated? 5-85
5.3.3.5 Determining Fish Lipid using the National Default Lipid Data Base 5-86
5.3.4 How Can Site-specific Organic Carbon Concentrations be Determined? ... 5-87
5.3.4.1 Overview of Freely Dissolved Normalization Process 5-89
5.3.4.2 Measuring DOC and POC 5-90
5.3.4.3 Determining DOC and POC Using a Literature or Database Search 5-92
5.3.4.4 How Should Organic Carbon Data Be Evaluated? 5-93
5.3.4.5 Determining Organic Carbon Concentrations using the National DOC/POC Database 5-95
REFERENCES 5-98

Appendix 5A - Internet-Accessible Databases Containing Lipid Content Data 5-103
Appendix 5B - Internet-Accessible Databases Containing Organic Carbon Data 5-109
Appendix 5C - BSAFs for PCB congeners in Green Bay and Upper Hudson River 5-113
Appendix 5D - Lipid Content of Aquatic Organisms 5-119

ED_006490_00009970-00012

TABLES

Table 2-1. Strengths and Limitations of the Methods for Deriving Site-specific BAFs (SS BAFs) 2-16

Table 3-1. Illustrative Bioaccumulation Factor Sampling Design Structures. Uncertainties Associated With Design Structures Are Ecosystem Specific 3-26

Table 3-2. Waterbody Type as Indicator of Temporal Concentration Variability 3-28

Table 3A-1. Bootstrap Results for PCB Congener 149 Forage Fish BAF: 90% Confidence Limit Ratio (Upper Confidence Limit/Lower Confidence Limit) as a Function of the Number of Fish and Water Samples. Smaller Ratios Indicate Less Uncertainty 3-72

Table 3A-2. Taylor Series Approximation of Confidence Limits for Baseline BAFs in Green Bay Zone 3 3-77

Tables 3C-1. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 2 Fish and 2 Water Samples. 3-88

Tables 3C-2. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 4 Fish and 4 Water Samples. 3-89

Tables 3C-3. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 6 Fish and 6 Water Samples. 3-89

Table 3C-4. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 2 Fish and 4 Water Samples. 3-90

Table 3C-5. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 4 Fish and 2 Water Samples 3-90

Table 4-1. Some Illustrative Biota–Sediment Accumulation Factor Sampling Design Structures. 4-31

ED_006490_00009970-00013

TABLES (CONTINUED)

Table 4-2. Number of 3-inch Diameter Cores Required to Composite 500 mL of Sediment 4-45

Table 4-3. Validation Statistics for Method 2: Ratio of Baseline $BAF_{predicted}$/Baseline $BAF_{measured}$ for all (combined) sampled fish species 4-49

Table 4-4. Geometric Mean Regression Equations (log $_{socw}$ = A log K_{OW}+ B) for Polychlorinated Biphenyls (PCBs) and Chlorinated Pesticides 4-53

Table 4-5. Average $_{socw}$ / K_{ow} Ratios for Three Different Ecosystems 4-59

Table 4B-1. 90% Confidence Interval Ratios for BSAF as Function of the Variability in Chemical Concentrations in Sediment 4-81

A. Chemical Concentrations Measured in 2 Fish Samples 4-81
B. Chemical Concentrations Measured in 4 Fish Samples 4-81
C. Chemical Concentrations Measured in 6 Fish Samples 4-81
D. Chemical Concentrations Measured in 10 Fish Samples 4-82
E. Chemical Concentrations Measured in 30 Fish Samples 4-82

Table 4B-2. 90% Confidence Interval Ratios for $_{socw}$ as Function of the Variability in Chemical Concentrations in Sediment 4-84

A. Chemical Concentrations Measured in 2 Water Samples 4-84
B. Chemical Concentrations Measured in 4 Water Samples 4-84
C. Chemical Concentrations Measured in 6 Water Samples 4-84
D. Chemical Concentrations Measured in 10 Water Samples 4-85
E. Chemical Concentrations Measured in 30 Water Samples 4-85

Table 4B-3. 90% Confidence Interval Ratios for Method 2 BAF Predictions for PCB Congener 149 as a Function of the Variability in Chemical Concentrations in Sediment ... 4-90

A. Chemical Concentrations Measured in 2 Fish and 2 Water Samples 4-90
B. Chemical Concentrations Measured in 4 Fish and 4 Water Samples 4-90
C. Chemical Concentrations Measured in 6 Fish and 6 Water Samples 4-90
D. Chemical Concentrations Measured in 10 Fish and 10 Water Samples 4-91
E. Chemical Concentrations Measured in 30 Fish and 30 Water Samples 4-91

Table 5-1. Method 3a BSAF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River. 5-19

A. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to 3 Year Old Brown Trout in Zone 4 of Green Bay (GB BT3) 5-19

ED_006490_00009970-00014

TABLES (CONTINUED)

B. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to 4 Year Old Walleye in Zone 4 of Green Bay (GB W4) 5-19
C. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to 4 Year Old Walleye in Zone 1 of Green Bay (GB W4) 5-20
D. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to Largemouth Bass at Hudson River mile 189 (RM 189 LMB)..... 5-20
E. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to Largemouth Bass at Hudson River mile 194 (RM 194 LMB)..... 5-21

Table 5-2. TCDD Bioaccumulation Equivalency Factors (BEFs) Derived For Toxicologically Important PCDDs And PCDFs From Lakewide Averages Of Concentrations In Lake Ontario Lake Trout And Surface Sediment In Depositional Areas. 5-28

Table 5-3. Method 3b BEF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River..... 5-30

A. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to 3 Year Old Brown Trout in Zone 4 of Green Bay (GB BT3)..... 5-30
B. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to 4 Year Old Walleye in Zone 4 of Green Bay (GB W4) 5-30
C. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to 4 Year Old Walleye in Zone 1 of Green Bay (GB W4) 5-31
D. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to Largemouth Bass at Hudson River mile 189 (RM 189 LMB)..... 5-31
E. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to Largemouth Bass at Hudson River mile 194 (RM 194 LMB)..... 5-32

Table 5-4. Food Web Structure for National BAF Methodology (Flint, 1986; Gobas, 1993) 5-54

Table 5-5. Environmental Parameters and Conditions Used for Determining FCMs for the National BAF Methodology..... 5-54

Table 5-6. Food-Chain Multipliers for Trophic Levels (TLs) 2, 3, and 4 (Mixed Pelagic and Benthic Food Web Structure and $_{socw}$ / K_{ow} = 23) 5-55

Table 5-7. Validation Statistics for Method 4b: Ratio Between Predicted and Measured Baseline BAFs (Baseline $BAF_{predicted}$/Baseline $BAF_{measured}$) based on PCB concentration data from Green Bay, Lake Michigan and the Hudson River..... 5-66

ED_006490_00009970-00015

TABLES (CONTINUED)

Table 5-8. Summary Statistics: Differences Between Log Baseline BAFs Predicted with Method 4b and Log Baseline BAFs Measured from Lake Ontario (Oliver and Niimi, 1988) for Chemicals with Log K_{ow} Exceeding 4 5-67

Table 5-9. BAFs and Baseline BAFs Confidence Limit Ratios (CLRs) for Adult alewife, Age 4 walleye, and Age 10 carp in Green Bay (All Zones Combined) 5-78

Table 5-10. Evaluation Criteria for Lipid Data Sources 5-86

Table 5-11. Evaluation Criteria for Organic Carbon Data Sources 5-94

Table 5-12. National Default Values for POC and DOC in U.S. Fresh and Estuarine Surface Waters 5-97

Table 5C-1. BSAFs for PCB congeners based on measurements made in Green Bay, Lake Michigan. 5-113

Table 5C-2. BSAFs for PCB congeners based on measurements made in the Hudson River. 5-115

Table 5D-1. Lipid Content of Aquatic Organisms Used to Derive National Default Values of Lipid Fraction (f_l) 5-119

ED_006490_00009970-00016

FIGURES

Figure 2-1 Framework for selection of methods for deriving national BAFs. 2-2

Figure 3A-1. Bootstrap distributions of forage fish and water concentrations, and BAFs based on resampling 20 fish and 20 water concentrations from Green Bay Zone 3 PCB 18 data 3-70

Figure 3A -2. Bootstrap distributions of forage fish and water concentrations, and BAFs based on resampling 2 fish and 20 water concentrations from Green Bay zone 3 PCB 18 data 3-71

Figure 3A-3. Bootstrap distributions of forage fish and water concentrations, and BAFs based on resampling 2 fish and 2 water concentrations from Green Bay zone 3 PCB 18 data 3-71

Figure 3A-4. Bootstrap resampling results for PCB congener 149 in Green Bay Zone 3 forage fish: 90% confidence interval ratios for BAF as a function of numbers of fish and water samples. 3-73

Figure 3A-5. Bootstrap resampling results for PCB congeners 18, 52, 149 and 180 in Green Bay Zone 3 forage fish: comparison of mean percent bias of BAF as a function of the numbers of water samples. 3-74

Figure 3A-6. Bootstrap resampling results for PCB congener 149 in Green Bay Zone 3 forage fish: Root mean square error (RMSE) for BAF as a function of numbers of fish and water samples. 3-75

Figure 3C-1. Comparison of Monte Carlo and Bootstrap results: Ratio of 90% confidence limits for BAF as a function of numbers of fish and water samples for PCB congener 149 in Green Bay Zone 3 predator fish 3-84

Figure 3C-2. Comparison of Monte Carlo and Bootstrap results: Ratio of 90% confidence limits for BAF as a function of numbers of fish and water samples for PCB congener 149 in Green Bay Zone 3 forage fish. 3-86

Figure 3C-3. Monte Carlo results for PCB congener 149 in Green Bay Zone 3 predator fish: Ratio of 90% confidence limits for BAF as a function of correlation between biota and water concentrations 3-87

ED_006490_00009970-00017

FIGURES (CONTINUED)

Figure 3D-1. (A) Daily chemical concentrations in the model river segment. (B) Daily chemical concentrations in piscivorous fish for chemicals with log K_{OW}s of 2, 3, 4, and 9. 3-94

Figure 3D-2. Ratio of the 10th to 90th percentile bioaccumulation factors (BAFs) for field-sampling designs. 3-96

Figure 4-1. The sediment-water concentration quotient ($_{socw}$) for three different chemical loading scenarios. 4-26

Figure 4-2. Predicted BAFs using Method 2 and 4b plotted against measured baseline BAFs for different sampling locations and fish species for the Green Bay (species and zone) and Hudson River (species and river mile) ecosystems. 4-48

Figure 4-3. Sediment-water column concentration coefficient ($_{socw}$) for PCBs in five different geographical zones in Green Bay, Lake Michigan 4-54

Figure 4-4. Average sediment-water column concentration coefficients ($_{socw}$) for individual PCB congeners across the five different geographical zones in Green Bay, Lake Michigan 4-55

Figure 4-5. Sediment-water column concentration coefficients ($_{socw}$) for PCBs at river miles 189 and 194 4-56

Figure 4A-1. Ratio of the 10th to 90th percentile biota–sediment accumulation factor (BSAF) for field-sampling designs 4-74

Figure 4B-1. 90% confidence interval ratio for BSAF as function of number of sediment samples. 4-80

Figure 4B-2. 90% confidence interval ratio for $_{socw}$ as function of number of sediment samples. 4-83

Figure 4B-3. 90% confidence interval ratio for PCB-180 Method 2 BAFs as a function of the number of sediment samples 4-86

Figure 4B-4. 90% confidence interval ratio for four PCB congener Method 2 BAFs as a function of the number of sediment samples. 4-87

Figure 4B-5. 90% confidence interval ratio for PCB-149 Method 2 BAFs as a function of the number of sediment samples and variability of chemical concentrations in sediment. 4-89

ED_006490_00009970-00018

FIGURES (CONTINUED)

Figure 4B-6. 90% confidence interval ratio for PCB-52 Method 2 BAFs as a function of concentration correlations 4-93

Figure 5-1. Decision framework for selecting a site-specific BAF derivation method based on BSAFs 5-4

Figure 5-2. White sucker BSAFs (kg organic carbon/kg lipid) from six ecosystems plotted against BSAFs (kg organic carbon/kg lipid) for white sucker from sampling station 01208869. 5-8

Figure 5-3. BSAFs (kg organic carbon/kg lipid) for PCDD/Fs with nonzero mammalian toxicity equivalence factors (TEFs) from four ecosystems plotted against BSAFs (kg organic carbon/kg lipid) for 6 year old lake trout from Lake Michigan 5-9

Figure 5-4. BSAFs (kg organic carbon/kg lipid) for PCBs from Green Bay, Hudson River, and Detroit River (Leadley et al. 1998) plotted against BSAFs (kg organic carbon/kg lipid) for 6 year old lake trout from Lake Michigan 5-10

Figure 5-5. BSAFs (kg organic carbon/kg lipid) for PCBs and PCDD/Fs from Lake Ontario (USEPA, 1995) and Tokyo Bay (Naito et al. 2003) plotted against BSAFs (kg organic carbon/kg lipid) for 6 year old lake trout from Lake Michigan 5-11

Figure 5-6. BSAFs (kg organic carbon/kg lipid) for PCBs from Green Bay and the Hudson River plotted against BSAFs (kg organic carbon/kg lipid) extrapolated from Lake Michigan (6 year old lake trout) using Method 3a. 5-22

Figure 5-7. BSAFs (kg organic carbon/kg lipid) for PCBs from Green Bay and the Hudson River plotted against BSAFs (kg organic carbon/kg lipid) estimated from Lake Michigan (6 year old lake trout) BEFs using Method 3b. 5-33

Figure 5-8. Relationship between baseline BAFs measured at Bayou d' Indie and BAFs predicted using Method 4a 5-43

Figure 5-9. The FCMs determined for the national BAF methodology for trophic levels 2 through 4 5-56

Figure 5-10. $BAF^{t}_{i,T}$ s and Baseline BAFs for PCB congener 149 (2,2',3,4',5',6-hexachlorobiphenyl) (±1 sd) for adult alewife for different spatial zones in Green Bay. 5-77

Figure 5-11. Box plots comparing baseline (TL 3 or 4 B) and field-measured (TL 3 or 4 F) BAFs for six PCB congeners obtained from Green Bay, Lake Ontario, and Hudson River ecosystems for 13 fish species 5-80

ED_006490_00009970-00019

FIGURES (CONTINUED)

Figure 5-12. Illustration of how the freely dissolved fraction calculated using equation 3-12 varies as a function of K_{ow}, for varying concentrations of DOC and POC. 5-90

ED_006490_00009970-00020

LIST OF ACRONYMS, SYMBOLS AND NOTATIONS

AWQC	Ambient water quality criteria
BAF	Bioaccumulation factor
BAF_T^t	Bioaccumulation factor based on total concentrations in tissue and water
BAF_l^{fd}	BAF, lipid normalized and based on freely dissolved chemical in water
BAF_L^{fd}	Baseline BAF, lipid normalized and based on freely dissolved chemical in water
BCF	Bioconcentration factor
BCF_l^{fd}	BCF, lipid normalized and based on freely dissolved chemical in water
BCF_L^{fd}	Baseline BCF, lipid normalized and based on freely dissolved chemical in water
BCF_T^t	Bioconcentration factor based on total concentrations in tissue and water
BEF	Bioaccumulation equivalency factor
BMF	Biomagnification factor
BSAF	Biota-sediment accumulation factors
BW	Human body weight
C	Concentration
superscript *fd*	Freely dissolved chemical
superscript *t*	Total chemical
subscript *w*	In water
subscript *soc*	In sediment organic carbon
subscript *t*	In tissue
subscript *l*	In lipid
subscript *r*	Reference chemical
subscript *k*	Individual chemical of interest
subscript *i*	In organism at trophic level *i*
C_w^t	C of total chemical in water
C_w^{fd}	C of chemical freely dissolved in water
C_s	C of chemical in sediment
C_{soc}	C of chemical in sediment organic carbon
C_l	C of chemical in lipid
C_t	C of chemical in the specified wet tissue
CDC	U.S. centers for disease control and prevention
CSFII	Continuing survey of food intake by individuals
CWA	Clean Water Act
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
DI	Drinking water intake
DOC	Dissolved organic carbon
EPA	Environmental Protection Agency
f_{fd}	Fraction freely dissolved
f_l	Fraction lipid
f_{oc}	Fraction organic carbon in suspended solids
f_{soc}	Fraction organic carbon in sediment

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LIST OF ACRONYMS, SYMBOLS AND NOTATIONS (CONTINUED)

FCM	Food chain multiplier
FI_i	Fish intake at trophic level *i*
GLI	Great Lakes Water Quality Initiative
IARC	International Agency for Research on Cancer
IRIS	Integration Risk Information System
kg	Kilogram
K_{ow}	n-Octanol-Water Partition Coefficient
L	Liter
MCL	Maximum Contaminant Level
mg	Milligrams
ml	Milliliters
NFCS	Nationwide Food Consumption Survey
NOEL	No Observed Effect Level
NPDES	National Pollutant Discharge Elimination System
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
POC	Particulate organic carbon
RDA	Recommended Daily Allowance
R*f*D	Reference dose
RSC	Relative source contribution to account for nonwater sources of exposure
SAB	Science Advisory Board
$_{socw}$	Sediment-water concentration quotient
$D_{k/r}$	Ratio between values of $_{socw}$ for reference chemical and chemical of interest *k*
SDWA	Safe Drinking Water Act
STORET	Storage Retrieval
TMDL	Total Maximum Daily Load
TSD	Technical Support Document
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
WQBEL	Water Quality-Based Effluent Limits

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1. INTRODUCTION

This Technical Support Document (TSD) provides guidance on different approaches that investigators can take to develop site-specific bioaccumulation factors (BAFs) that are representative of the bioaccumulation potential at a given location, and the factors that should be considered when selecting an approach for developing a site-specific BAF in a given situation. This TSD should not be used alone to derive BAFs, but rather should be used in conjunction with the *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (USEPA, 2000) and *TSD Volume 2: Development of National Bioaccumulation Factors* (USEPA, 2003). The intended audience for this TSD includes State and Tribal water quality staff scientists or risk assessors ("investigators") who are responsible for deriving State or Tribal water quality standards, stakeholders interested in developing site-specific BAFs, and other users interested in site-specific bioaccumulation issues for other applications. The 2000 Human Health Methodology for deriving ambient Water Quality Criteria will provide more flexibility for decision-making at the state, tribal and EPA regional levels. It is most likely that the methodology will result in more stringent water quality criteria for chemicals that bioaccumulate and generally similar values for chemicals that do not.

The bioaccumulation methodology used in the 2000 Human Health Methodology encourages developing site-specific BAFs because EPA recognizes that BAFs vary not only between chemicals and trophic levels, but also among different ecosystems and waterbodies; that is, among sites. The bioaccumulation potential of a chemical can be affected by various site-specific physical, biological, and chemical factors:

- water temperature and dissolved oxygen concentration;
- sediment-water disequilibria;
- organism health, physiology and growth rate;
- food chain structure;
- food quality; and
- organic carbon composition.

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National average BAF value for a given chemical and trophic level may not provide the most accurate estimate of bioaccumulation for certain waterbodies in the United States. At a given location, the BAF for a chemical may be higher or lower than the national BAF, depending on the nature and extent of site-specific influences. In addition, the fish consumption habits of the local human population will guide the selection of the target species for which the investigator develops site-specific BAFs.

The goal in deriving site-specific BAFs is to determine the most accurate estimates of bioaccumulation feasible for each site. In the absence of site-specific data, EPA believes that national BAFs are broadly applicable to sites throughout the United States and can be applied to achieve an acceptable degree of accuracy when estimating bioaccumulation potential at most sites. National BAFs are derived using a methodology intended to produce the most accurate national average values for BAFs at each trophic level. The investigator should view the derivation of site-specific BAFs as a process to improve upon the accuracy of the national BAFs for a particular site. EPA expects that in most instances, the derivation of site-specific BAFs will be motivated by some knowledge or expectation that unique site-specific factors may cause BAFs to diverge from the national values. These factors include (for example): fish consumption patterns that are substantially different than national averages; species of aquatic organisms that have not been previously sampled or for which trophic level or feeding preference is unknown; and sediment-water chemical distribution, tissue lipid content or DOC concentration significantly different than the values assumed in the national methodology. In cases such as these, the derivation of site-specific BAFs would likely improve the accuracy of bioaccumulation estimates and, ultimately, the AWQC for the chemical of concern at that site. The issue of what range of sites the national BAFs are intended to represent, and the potential variation in BAF values between sites, is considered in greater detail in TSD Volume 2 (USEPA, 2003).

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1.1 BACKGROUND

The *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000)* (USEPA, 2000) presented technical guidance and the steps that EPA follows to derive new and revised national recommended ambient water quality criteria (AWQCs) for the protection of human health under Section 304(a) of the Clean Water Act. Water quality criteria define the maximum levels of a pollutant necessary to protect designated uses in ambient waters. For chemicals that bioaccumulate, water quality criteria also describe the maximum advisable concentration of a chemical in freshwater and estuarine fish and shellfish tissue to protect consumers of fish and shellfish among the general population. The 2000 Human Health Methodology included guidance on chemical risk assessment, exposure, and bioaccumulation. To supplement the 2000 Human Health Methodology, EPA is developing a series of Technical Support Documents (TSDs) on Risk Assessment, Exposure Assessment, and Bioaccumulation. The first volume, *Volume 1: Risk Assessment* (EPA-822-B-00-005), was published with the 2000 Human Health Methodology in October of 2000.

In 2003, the EPA published a second technical support document, *Volume 2: Development of National Bioaccumulation Factors* (EPA-822-R-03-030), to accompany the 2000 Human Health Methodology. That document focused on the technical components of the 2000 Human Health Methodology that pertain to the development of national bioaccumulation factors for use in deriving national recommended ambient water quality criteria for protecting human health. A national bioaccumulation factor (National BAF_i)[1] is a mean BAF, based on concentrations of total chemical in wet tissue and water, for a specific trophic level "*i*". It is adjusted for the consumption-weighted average lipid content of commonly consumed aquatic organisms in that trophic level and the nationwide average organic carbon concentration in ambient waters. In this document we refer to national BAFs as plural, because the human population usually consumes aquatic organisms from more than one trophic level and, therefore, EPA develops a national BAF for each of these trophic levels.

[1] In TSD Volume 2, a slightly different symbol (National $BAF_{TL\,n}$) was used for national bioaccumulation factors. The two symbols are equivalent.

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For those unfamiliar with EPA's methodology for assessing chemical bioaccumulation (USEPA, 2003), it is useful to review how BAFs are factored into the calculation of recommended national AWQCs for the protection of human health. Equation 1-1 (below) is the generalized AWQC formula for noncancer effects. In this equation, trophic-level specific BAF_is are in the denominator, along with information on the amount of fish of each trophic level (i) consumed on a daily basis (FI_i), to estimate human exposure to contaminants through the aquatic food web (USEPA, 2000).

$$AWQC = RfD \cdot RSC \cdot \left(\frac{BW}{DI + \sum_{i=2}^{4} \left(FI_i \cdot BAF_i \right)} \right) \qquad \text{(Equation 1-1)}$$

where:

AWQC = ambient water quality criterion (mg chemical/L water)

R*f*D = reference dose for noncancer effects (mg/kg/day)

RSC = relative source contribution to account for nonwater sources of exposure

BW = human body weight (kg)

DI = drinking water intake (L/day)

FI_i = fish intake (kg/day) at trophic level i (i = 2, 3, 4)

BAF_i = bioaccumulation factor (L/kg) at trophic level i (i = 2, 3, 4) based on concentrations of total chemical in wet tissue and water

For contaminants that bioaccumulate extensively, such as hydrophobic nonionic organic chemicals, researchers report BAF_i values of 10^3 to 10^7 for aquatic ecosystems. For these chemicals, inspection of Equation 1-1 reveals that the AWQC will be inversely proportional to the BAF. The EPA's approach to estimating uptake into fish and shellfish emphasizes the use of bioaccumulation factors, which account for chemical accumulation from all potential exposure routes (e.g., food, sediment, and water) that may be important in determining the chemical accumulation in the organism's body. As noted in Section 1.2 of the 2000 Human Health Methodology, however, EPA and State/Tribal decision-makers retain the discretion to use different, scientifically defensible, methodologies to develop human health criteria on a case-by-case basis that differ from this Methodology (i.e., the use of BAFs and Equation 1-1) where

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appropriate. For example, in January 2001, EPA published ambient water quality criteria (AWQC) recommendations for methylmercury for the protection of people who eat fish and shellfish. This criterion, 0.3 mg methylmercury/kg fish tissue wet weight, marked EPA's first issuance of a water quality criterion expressed as a fish and shellfish tissue value rather than as an ambient water column value (USEPA, 2009).

1.2 SCOPE OF DOCUMENT

EPA's approach for deriving national BAFs includes separate procedures for different types of chemicals (e.g., nonionic organic, ionic organic, inorganic, and organometallic). For the purposes of the 2000 Human Health Methodology, nonionic organic chemicals are defined as organic compounds that do not ionize substantially in natural bodies of water. These chemicals are also referred to as "neutral" or "nonpolar" organics in the scientific literature. TSD Volumes 2 and 3 focus primarily on calculation procedures for determining BAFs for nonionic organic chemicals that bioaccumulate in the lipids of fish and partition to organic carbon in water due to the hydrophobicity of the chemical. BAF calculation procedures and theories presented in both documents are based on this partitioning behavior which does not apply to ionic organic chemicals, in their ionized form, or inorganic and organometallic compounds. Therefore, the BAF calculation procedures presented in this document are applicable mainly to nonionic organic chemicals. The procedures for estimating the bioaccumulation of nonionic organic chemicals are generally better developed than those for other types of chemicals. The conditions under which these procedures can be applied and the limitations associated with their application must be understood for their proper application, and will be discussed further in Section 2.

Ionic chemicals are considered separately when deriving national BAFs because the environmental partitioning behavior of the anionic or cationic species of these chemicals in aquatic systems is much different from those of their neutral (un-ionized) counterparts. Ionic organic chemicals are considered to include those chemicals that contain functional groups which can either readily donate protons (e.g., organic acids with hydroxyl, carboxylic, and sulfonic groups) or readily accept protons (e.g., organic bases with amino and aromatic

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heterocyclic nitrogen groups). The neutral species of ionic organic chemicals are thought to behave in a similar manner as nonionic organic compounds (e.g., partitioning to lipids and organic carbon as a function of hydrophobicity). However, ionic organic chemicals undergo ionization in ambient water, the extent of which depends on the pH of the water and the pKa of the chemical (see Section 5.5 of the 2000 Human Health Methodology for guidance). The ionized chemical species exhibit a considerably more complex partitioning behavior than non-ionic organic chemicals, involving multiple partitioning mechanisms (e.g., ion exchange, electrostatic, and hydrophobic interactions (Jafvert et al., 1990; Jafvert 1990; Schwarzenbach, et al., 1993). As discussed in Section 5.5 of the 2000 Human Health Methodology, procedures for deriving BAFs for these chemicals differ depending on the extent to which the fraction of the total chemical is likely to be represented by the ionized (cationic, anionic) species in surface waters.

Inorganic and organometallic chemicals are also considered separately from nonionic organic chemicals, due to several important factors. These chemical groups include:

- inorganic minerals,
- other inorganic compounds and elements,
- metals,
- metalloids, and
- organometallic compounds.

As discussed in Section 5.6 of the 2000 Human Health Methodology, the derivation of BAFs for inorganic and organometallic chemicals differs in several ways from procedures for nonionic organic chemicals. First, these chemicals do not partition to lipids and organic carbon in ambient waters as do nonionic organic chemicals. Second, the bioavailability of inorganics and organometallics in water tends to be chemical-specific. Third, at the present time there are no general bioaccumulation models that can be used to predict BAFs for inorganic and organometallic chemicals as a whole, unlike the hydrophobicity-based, lipid and organic carbon partitioning models that are available for nonionic organic chemicals. The procedures presented in this TSD (Volume 3), which are based on the partitioning behavior of nonionic organic

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chemicals to the lipids in fish and organic carbon in water, are not applicable for calculating BAFs for ionic organic chemicals (when ionized) and inorganic or organometallic chemicals.

1.3 SITE-SPECIFIC BIOACCUMULATION FACTORS (SS BAFs)

There are two general approaches for deriving site-specific BAFs. The preferred approach is to calculate site-specific BAFs or biota-sediment accumulation factors (BSAFs) from data gathered in the site(s) of interest. BAFs derived from data obtained from samples of tissue and water collected at the site - referred to as "field-measured BAFs" - are the most direct measures of bioaccumulation. For nonionic organic chemicals (and ionic organic chemicals that behave similarly), the investigator can also predict site-specific BAFs from BSAFs. BSAFs are similar to field-measured BAFs because the concentration of a chemical in biota is calculated from the results of the analysis of samples of tissue and sediment collected at the site. BSAFs also reflect an organism's exposure through all relevant exposure routes. EPA prefers field-measured BAFs and BSAFs over other methods of determining site-specific BAFs because they inherently account for all biotic and abiotic factors that affect bioaccumulation in a waterbody. EPA encourages the States, Territories and authorized Tribes to develop field-measured BAFs and BSAFs whenever possible.

The second general approach is to estimate site-specific BAFs indirectly using one of the other methods described in TSD Volume 2 (USEPA, 2003). These methods include:

- recalculating site-specific BAFs from baseline[3] BAFs,
- extrapolating site-specific BAFs from BSAFs, or
- predicting BAFs using laboratory-measured bioconcentration factors (BCFs) or octanol-water partition coefficients (K_{ow}s) coupled with food chain multipliers.

[3] For nonionic organic chemicals (and certain ionic organic chemicals to which similar lipid and organic carbon partitioning behavior applies), the baseline BAF is the ratio between the chemical concentration in the lipid fraction of tissue and the concentration of chemical freely dissolved in water.

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Although these methods are the same as those described in TSD Volume 2, EPA expects that variations of these methods, described in this TSD, may be used to derive site-specific BAFs. EPA encourages those deriving site-specific BAFs to use as many of these methods as possible and then compare the results, applying judgment to select the best estimate. The guidance discusses benefits and limitations of each approach (see Table 2-1) and EPA recommends consideration of these when choosing method(s). EPA believes that the additional guidance provided in this TSD will help to ensure that site-specific bioaccumulation factors are accurate and defensible, whether they are determined directly by field measurement or indirectly by estimation methods.

The remainder of this document is organized into four sections:

- Section 2 discusses the definition of a site, and introduces the different methods that the investigator can use to derive site-specific BAFs.
- Section 3 discusses the derivation of field-measured site-specific BAFs, from data obtained on samples of tissue and water collected at the site. This section also provides guidance to the investigator for planning a field study to measure chemical concentrations in water and fish tissue.
- Section 4 presents the derivation of site-specific BAFs predicted from BSAFs measured at the site, and also provides guidance related to measuring chemical concentrations in sediment.
- Section 5 presents the other methods for deriving site-specific BAFs based upon applying one of the other methods described in TSD Volume 2 (USEPA, 2003). This section also discusses the adjustment of lipid and organic carbon values used in estimating the site-specific BAFs, and the alternatives that the investigator can use to determine site-specific values of lipid and organic carbon.

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1.4 GLOSSARY

The following terms and their definitions are used throughout this document, and were based upon the glossary provided in TSD Volume 2 (USEPA, 2003). Differences between this TSD and Volume 2 are noted below.

Bioaccumulation. The net accumulation of a chemical by an aquatic organism as a result of uptake from all environmental sources (water, sediment and food).

Bioaccumulation factor (BAF_i **).** The ratio of the concentration of a chemical in the tissue of an aquatic organism to its concentration in water, in situations where both the organism and its food are exposed and the ratio does not change substantially over time. The subscript *i* indicates that a BAF_i is trophic level specific; this subscript was not used in TSD Volume 2. Several forms of the BAF_i are used in this document:

Total bioaccumulation factor ($BAF_{i,l}^{t}$). A BAF based on the ***total*** concentration of chemical in the organism and the water. The total concentration of the chemical in the organism includes that in either a specific tissue (e.g., fillet) or the whole organism and is based on wet tissue weight. The total concentration of the chemical in water includes the chemical associated with particulate organic carbon, chemical associated with dissolved organic carbon, and chemical freely dissolved in the water. The $BAF_{i,l}^{t}$ is expressed in liters per kilogram, and is trophic level specific. The subscript *i* was not used in TSD Volume 2.

Baseline bioaccumulation factor (Baseline BAF_i or $BAF_{i,L}^{fd}$). For nonionic organic chemicals (and certain ionic organic chemicals to which similar lipid and organic carbon partitioning behavior applies[4]), a BAF that is based on the concentration of the chemical in the lipid fraction

[4] As discussed in TSD Volume 2, baseline and lipid-normalized BAFs for certain ionic organic chemicals can be derived using methods developed for nonionic organic chemicals, which rely on lipid and organic carbon partitioning theory. In these cases, similar lipid and organic carbon partitioning behavior should be known or inferred (i.e., based on negligible ionization) for the ionic chemical in question. If the relative extent of ionization that is likely to occur at pH ranges that are typical of U.S. surface waters is negligible (see the 2000 Human Health Methodology for guidelines on this determination), and if the un-ionized form of the ionic chemical behaves like a nonionic organic chemical, in which lipid and organic carbon partitioning controls the behavior of the chemical, then the chemical can be treated essentially as a nonionic chemical for the purposes of determining site-specific BAFs.

ED_006490_00009970-00031

of tissue within an organism and the concentration of chemical freely dissolved in water. The baseline BAF_i is trophic level specific, although the subscript ***i*** was not used in TSD Volume 2. The baseline BAF_i is expressed in liters per kilogram of lipid.

Field-measured bioaccumulation factor. A $BAF_{i,T}^{t}$ derived from analysis of tissue and water samples collected from the field. For moderately to highly hydrophobic chemicals, (log $K_{ow} > 4$) it is usually preferable to measure a $BAF_{i,l}^{fd}$ instead.

Lipid-normalized and freely dissolved-based bioaccumulation factor ($BAF_{i,l}^{fd}$ **).** For nonionic organic chemicals (and ionic organic chemicals with similar lipid and organic carbon partitioning behavior), a BAF that is based on the lipid-normalized concentration of a chemical in tissue of an organism and the concentration of the chemical freely dissolved in water. The $BAF_{i,l}^{fd}$ is expressed in liters per kilogram of lipid. The subscript *i* was not used in TSD Volume 2.

National trophic-level specific bioaccumulation factor (National BAF_i**).** A BAF based on nationwide average lipid content for trophic level *i* and nationwide average organic carbon in ambient waters. The national BAF_i is expressed in liters per kilogram wet tissue. In TSD Volume 2, the symbol National $BAF_{TL\,n}$ was used for this term.

Bioconcentration. The net accumulation of a chemical by an aquatic organism as a result of uptake directly from the ambient water only, through gill membranes or other external body surfaces.

Bioconcentration factor (BCF**).** The ratio of the concentration of a chemical in the tissue of an aquatic organism to its concentration in water, in situations where the organism is exposed through the water only and the ratio does not change substantially over time.

Total bioconcentration factor (BCF_T^t **).** A BCF based on the *total* concentration of chemical in the organism and the water. The total concentration of the chemical in the organism includes that in either a specific tissue or the whole organism and is based on wet tissue weight. The total concentration of the chemical in water includes the chemical associated with particulate organic carbon, the chemical associated with dissolved organic carbon, and the chemical freely dissolved in the water. A BCF is often referred to as a "laboratory-measured BCF" because it can be measured only in the laboratory. A BCF reflects only the accumulation of a chemical through the organism's exposure to water. The BCF_T^t is expressed in liters per kilogram.

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Baseline bioconcentration factor (Baseline BCF or BCF_L^{fd} **).** For nonionic organic chemicals (and certain ionic organic chemicals to which similar lipid and organic carbon partitioning behavior applies), a BCF that is based on the concentration of chemical freely dissolved in water and the concentration of the chemical in the lipid fraction of tissue. The baseline BCF is expressed in liters per kilogram of lipid.

Lipid-normalized and freely dissolved-based bioconcentration factor (BCF_l^{fd} **).** The ratio of the lipid-normalized concentration of a chemical in tissue of an organism to the concentration of the chemical freely dissolved in water, in situations where both the organism is exposed through water only and the ratio does not change substantially over time. The BCF_l^{fd} is expressed in liters per kilogram of lipid.

Biomagnification. The increase in concentration of a chemical in the tissue of organisms along a series of predator-prey associations, primarily through the mechanism of dietary accumulation. Biomagnification occurs across trophic (food chain) levels as opposed to bioaccumulation, which occurs within a trophic level.

Biomagnification factor (BMF_i**).** The ratio (unitless) of the concentration of a chemical in a predator organism at trophic level *i* to the concentration of the chemical in the tissue of its prey organism at the next lowest trophic level for a given waterbody and chemical exposure. In TSD Volume 2, the symbol $BMF_{TL\,n}$ was used for this term.

Biota-sediment accumulation factor ($BSAF_i$**).** For nonionic organic chemicals (and certain ionic organic chemicals to which similar lipid and organic carbon partitioning behavior applies), the $BSAF_i$ is the ratio of the lipid normalized concentration of a chemical in tissue of an aquatic organism to its organic carbon normalized concentration in surface sediment. $BSAF_i$ is only predictive of bioaccumulation for moderately to highly hydrophobic nonionic organic chemicals when: (1) the ratio does not change substantially over time; (2) both the organism and its food are exposed; and (3) the surface sediment is representative of average surface sediment in the vicinity of the organism. $BSAF_i$ is expressed in kilograms of sediment organic carbon per kilogram of lipid. The subscript *i* was not used in TSD Volume 2.

Bioaccumulation Equivalency Factor ($BEF_{k/r}$). For nonionic organic chemicals (and certain ionic organic chemicals to which similar lipid and organic carbon partitioning behavior applies), the $BEF_{k/r}$ is the ratio between the BSAF for a chemical *k* and the BSAF for another chemical *r*,

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when both BSAFs are measured in the same ecosystem.

Depuration. Loss of a chemical from an organism as a result of any active or passive physiological process.

Equilibrium. A thermodynamic condition under which a chemical's activity, or fugacity, is equal among all phases composing the system of interest. In systems at equilibrium, chemical concentrations in all phases will remain unchanged over time.

Food-chain multiplier (FCM_i). For nonionic organic chemicals (and certain ionic organic chemicals to which similar lipid and organic carbon partitioning behavior applies), the FCM_i is the unitless ratio of a baseline BAF for an organism at trophic level *i* to the baseline BCF (usually determined for organisms in trophic level one). The subscript ***i*** was not used in TSD Volume 2.

Foraging range. The area in which an individual organism normally feeds.

Freely dissolved concentration (C_w^{fd}). For nonionic organic chemicals, the concentration of the chemical that is dissolved in ambient water, excluding the portions sorbed onto particulate and dissolved organic carbon (POC and DOC). The freely dissolved chemical concentration is considered to represent the most bioavailable form of an organic chemical in water and, therefore, is the form that best predicts bioaccumulation.

Home range. The area to which an individual organism restricts most of its normal activities.

Hydrophilic. Chemicals having a great affinity to water. Hydrophilic chemicals are usually charged or have polar side groups to their structure that will attract water.

Hydrophobic. Lacking affinity for water; the extent to which a chemical avoids partitioning into the water phase. Moderately to highly hydrophobic organic chemicals ($\log K_{ow} > 4$) have a greater tendency to partition into nonpolar phases (e.g., lipid, organic carbon) than do hydrophilic chemicals.

Lipid-normalized concentration (C_l). The total concentration of a chemical in a tissue or whole organism divided by the fraction of that tissue or whole organism that is lipid.

***n*-Octanol-water partition coefficient (K_{ow}).** The ratio of the concentration of a chemical in the *n*-octanol phase to its concentration in the aqueous phase in an equilibrated two-phase system of *n*-octanol and water. This is usually expressed as $\log K_{ow}$, the base 10 logarithm of the *n*-octanol-water partition coefficient.

Sediment organic carbon-normalized concentration (C_{SOC}). For sediments, the total

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concentration of a contaminant in sediment divided by the fraction of organic carbon in sediment.

Sediment-water column concentration quotient ($_{socw}$). The ratio of the concentration of chemical in the sediment, on an organic carbon basis, to that in the water column, on a freely dissolved basis. $_{socw}$ when divided by the K_{ow} of the chemical provides a measure of the chemical's thermodynamic gradient between the sediment and the water column, for a given ecosystem. The sediment-water column concentration quotient is expressed in liters per kilogram of organic carbon.

Steady state. A condition reached by a system (e.g., an ecosystem composed of water, biota and sediment) when rates of chemical movement between phases and reactions within phases are balanced, so that concentrations of the chemical in the phases of the system are unchanged over time. A system at steady state is not necessarily at equilibrium; steady-state conditions often exist when some or all of the phases of the system have different activities or fugacities for the chemical.

Trophic Level. A trophic level of an organism is its position in a food chain. Levels are numbered according to how far particular organisms are along the chain from the primary producers (e.g., phytoplankton) at level 1, to herbivores (zooplankton; level 2), to predators (forage fish; level 3), to carnivores or top predators (level 4).

Uptake. Movement of chemical from the environment into an organism as the result of any active or passive process.

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2. HOW TO SELECT AN APPROACH FOR DERIVING SITE-SPECIFIC BAFs

This section provides guidance on selecting an approach (or approaches) for deriving site-specific BAFs from the alternatives recommended by EPA in this document. The guidance is intended to apply to all sites in the United States, and to each of the parties (States, Territories or authorized Tribes and other stakeholders) that may be interested in deriving site-specific BAFs. EPA recognizes that these parties may derive site-specific BAFs for different purposes, and may also have different views as to what constitutes a "site". The investigator should consider these institutional perspectives, in addition to other factors such as resource and schedule constraints, in conjunction with scientific preference when selecting an approach for deriving site-specific BAFs. State and Tribal decision makers also retain the discretion to use EPA's national BAFs, or scientifically defensible methodologies, including those discussed in this guidance and others, to develop site-specific BAFs, as appropriate. As a result, there is not a single approach that is preferable, or even applicable, for all sites. In each case the investigator should determine the hierarchy of preferred approaches based upon all of these considerations.

The methodology EPA uses to derive national BAFs for setting AWQCs for the protection of human health depends on the type of chemical (i.e., nonionic organic, ionic organic, inorganic, and organometallic). For a given chemical, the choice of a method for deriving a national BAF depends on several factors. These factors include the properties of the chemical of interest, the relative strengths and limitations of the BAF method, and the level of uncertainty associated with the bioaccumulation or bioconcentration measurements. Because selecting the most appropriate BAF method(s) for a given chemical and data set involves multiple evaluation steps, EPA developed a decision framework for deriving national BAFs (Figure 2-1). This framework illustrates the major steps and decisions that will ultimately lead to calculating a national BAF. Use of this framework leads to selection of one of six possible procedures (shown at the bottom of Figure 2-1) for deriving national BAFs. Each procedure includes those BAF derivation methods that are suitable for the class and properties of chemicals to which the procedure applies. The investigator should use the same framework to select appropriate methods for deriving site-specific BAFs.

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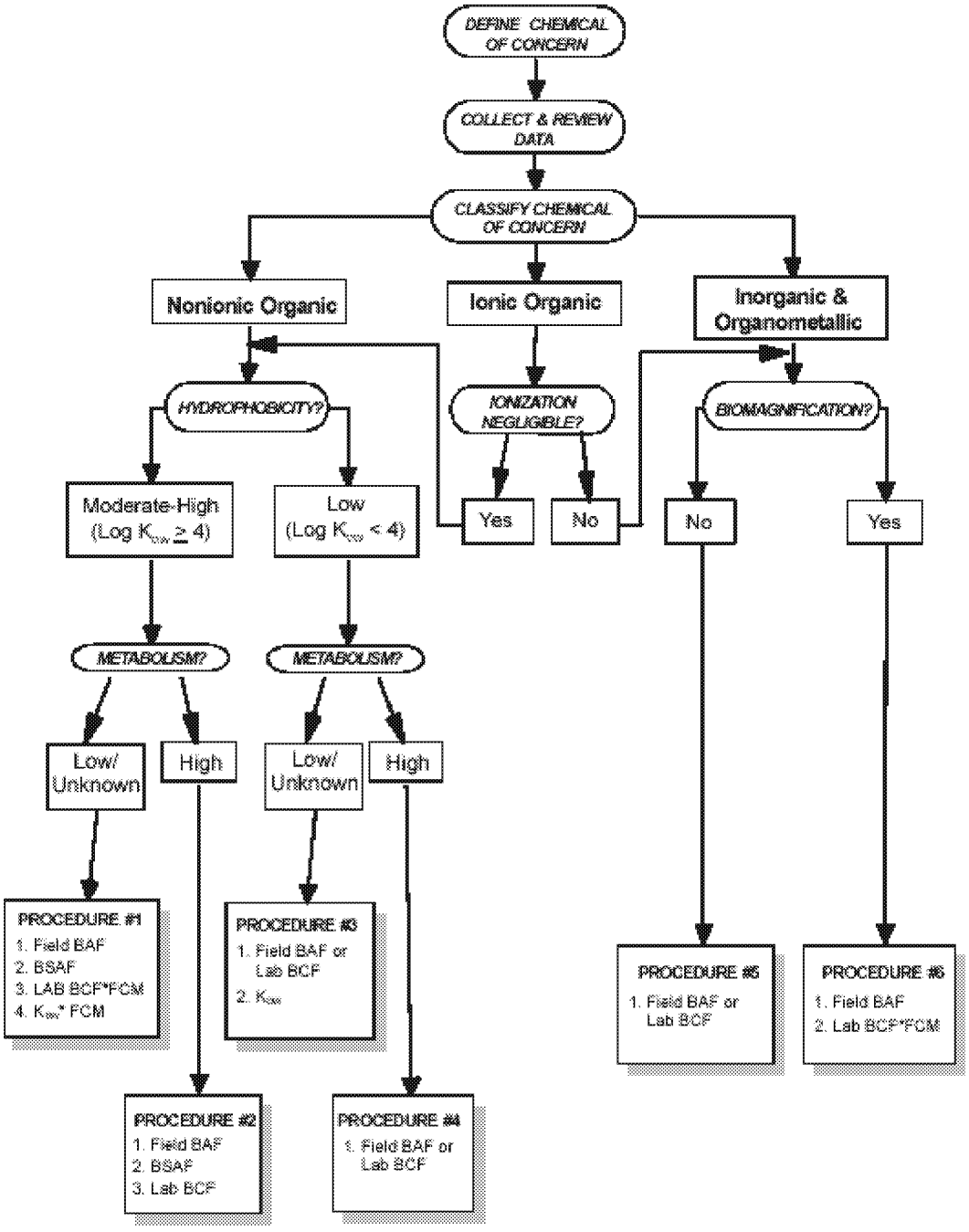

Figure 2-1. Framework for selection of methods for deriving national BAFs.

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The first step in the national BAF derivation framework involves precisely defining the chemical of concern. The purpose of this step is to ensure consistency between the form(s) of chemical used to derive national BAFs and the form(s) used as the basis of the health assessment (e.g., the reference dose or point of departure/uncertainty factor). Although this step is usually unambiguous for single chemicals that are stable in the environment, complications can arise when assessing chemicals that occur as mixtures or undergo complex transformations in the environment. The second step of the framework consists of collecting and reviewing data on bioaccumulation and bioconcentration. The third step involves classifying the chemical into one of three broadly defined categories: nonionic organic, ionic organic, and inorganic/ organometallic. This step is important because the BAF derivation methods presented in this document, and summarized in Section 2.5, are generally only applicable to nonionic organic chemicals and other chemicals with similar partitioning and bioaccumulation behavior. For the purposes of the 2000 Human Health Methodology, nonionic organic chemicals are defined as organic compounds that do not ionize substantially in natural bodies of water. These chemicals are also referred to as "neutral" or "nonpolar" organics in the scientific literature (Schwartzenbach et al., 1993; Mackay, 2001). Due to their neutrality, nonionic organic chemicals tend to associate with other neutral (or near neutral) compartments in aquatic ecosystems (e.g., lipid, organic carbon). Examples of nonionic organic chemicals which have been widely studied in terms of their bioaccumulation include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and furans, many chlorinated pesticides, and polycyclic aromatic hydrocarbons (PAHs).

Ionic organic chemicals are considered to include those chemicals that contain functional groups with exchangeable protons, such as: hydroxyl, carboxylic, sulfonic, and nitrogen (pyridine) groups and functional groups that readily accept protons such as amino and aromatic heterocyclic nitrogen (pyridine) groups. Ionic organic chemicals undergo ionization in water, the extent of which depends on pH and the pKa of the chemical. Because the ionized species of these chemicals behave differently from the neutral species, separate guidance is provided for deriving BAFs for ionic organic chemicals. Procedures for deriving national BAFs for ionic organic chemicals are provided in Section 5.5 of the 2000 Human Health Methodology.

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Inorganic and organometallic chemicals include:

- inorganic minerals,
- other inorganic compounds and elements,
- metals (e.g., copper, cadmium, chromium, zinc),
- metalloids (selenium, arsenic) and
- organometallic compounds (e.g., methylmercury, tributyltin, tetraalkyllead).

As discussed in Section 5.6 of the 2000 Human Health Methodology, the derivation of BAFs for inorganic and organometallic chemicals differs in several ways from procedures for nonionic organic chemicals. EPA does not consider the BAF derivation methods presented in this document (TSD Volume 3) to apply to most inorganic and organometallic chemicals.

Additional guidance on the first three steps of the framework is found in Section 5.3 of the 2000 Human Health Methodology. Once the chemical is classified into one of the three chemical categories, additional evaluation steps are necessary to determine which of the BAF procedures should be used to derive a national BAF. Again, the investigator should use the same framework to select appropriate methods for deriving site-specific BAFs.

2.1 BAF DERIVATION PROCEDURES FOR INORGANIC AND ORGANOMETALLIC CHEMICALS

For inorganic and organometallic chemicals, the primary factor to be evaluated is the likelihood that the chemical will undergo biomagnification in the food web. At present, evaluating the biomagnification potential for this group of chemicals is almost exclusively limited to analyzing empirical data on the importance of the aquatic food web (dietary) exposure and biomagnification in determining chemical concentrations in aquatic species. For example, available data indicate that methylmercury biomagnifies in aquatic food webs, whereas other chemicals in the inorganic and organometallic category do not routinely biomagnify (e.g., copper, zinc, lead). If biomagnification is considered to be likely, then field-measured BAFs are the preferred BAF method, followed by laboratory-measured BCF adjusted with an FCM. If biomagnification is determined to be unlikely, field-measured BAFs and laboratory-measured

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BCF are considered to be of equal utility for deriving national BAFs, all other factors being equal. Additional guidance on determining national BAFs for inorganic and organometallic chemicals is provided in Section 5.6 of the 2000 Human Health Methodology. It should be noted that metal bioaccumulation can vary substantially across organisms due to a number of factors including physiological differences and variation in mechanisms by which organisms take up, distribute, detoxify, store, and eliminate metals from their tissues.

EPA's *Framework for Metals Risk Assessment* (USEPA, 2007) outlines key principles about metals and describes how they should be considered in conducting human health and ecological risk assessments. Issues involving the bioavailability and bioaccumulation of metals in aquatic ecosystems are discussed in Chapter 5.2.5 of the *Framework*, while bioaccumulation and trophic transfer of metals are discussed in Chapter 5.2.5.4. Due to these issues, EPA does not consider the BAF derivation methods presented in this document (TSD Volume 3) to apply to most metals.

2.2 BAF DERIVATION PROCEDURES FOR IONIC ORGANIC CHEMICALS

For chemicals classified as ionic organic chemicals, the primary evaluation step involves estimating the relative extent of ionization and evaluating their partitioning behavior with lipids and organic carbon. This evaluation should include determining the relative extent of ionization that is likely to occur at pH ranges that are typical of the site water (see the 2000 Human Health Methodology for guidelines on this determination). If the relative extent of ionization is negligible, and if the unionized form of the ionic chemical behaves like a nonionic organic chemical (i.e., lipid and organic carbon partitioning controls the behavior of the chemical), then the chemical can be treated essentially as a nonionic chemical for the purposes of deriving site-specific BAFs. If ionization is considered potentially important, or if non-lipid and non-organic carbon mechanisms control the behavior of the chemical, then the ionic chemical is treated in the same way as inorganic and organometallic chemicals for deriving national BAFs. Additional guidance for deriving national BAFs for ionic organic chemicals is provided in Section 5.5 of the 2000 Human Health Methodology. Perfluorinated alkyl acids are an example of ionic organic chemicals. Some of these chemicals bioconcentrate and biomagnify in food webs via non-lipid

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mediated mechanisms; i.e., lipid and organic carbon partitioning behavior observed for nonionic organic chemicals does not apply. For the perfluorinated alkyl acids, Procedure 6 (Figure 2-1) would be used to derive national default BAFs.

2.3 BAF DERIVATION ASSUMPTIONS

The methods for deriving national and site-specific BAFs share a number of fundamental assumptions. First, EPA assumes that properly derived BAFs can provide a best estimate of chemical bioaccumulation under steady state (i.e., long-term) conditions that exist in the ecosystem.

The second major assumption associated with the use of BAFs for nonionic chemicals is that adjusting the BAF for the organism's lipid content and the chemical concentration that is freely dissolved removes much of the variability in BAFs across different species (within a trophic level) and across sites. This is the rationale for calculating baseline BAFs for nonionic organic chemicals. Section 4 of TSD Volume 2 (USEPA, 2003) provides the scientific basis for this assumption and a detailed discussion of baseline BAFs. EPA presumes that the residual variation in BAFs across different species and sites reflects other factors that influence bioaccumulation. These include:

- differences in chemical loading histories (i.e., sediment-water disequilibria);
- food web structure;
- organism health and physiology;
- water quality factors such as temperature; and
- food quality.

Each of these factors may vary across ecosystems and sites within an ecosystem.

A third major assumption with the use of any BAF is that the steady-state bioaccumulation of a chemical can be accurately predicted from a constant ratio of tissue to water concentration (i.e., the BAF is independent of exposure concentration). For nonionic

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organic chemicals, this assumption is generally supported by empirical and mechanistic evidence (i.e., uptake via passive diffusion; Kelly et al. 2004).

2.4 WHAT IS THE DEFINITION OF A SITE?

Investigators typically determine a site-specific BAF for a specific chemical, target species, and site. Each of these factors may influence the value of the site-specific BAF. The "site" refers to a spatial scale of interest smaller than the National level. Obviously, this definition encompasses a great range of spatial scales and different aggregations of waterbodies. A site can be a State, Territory or authorized Tribe; all surface waterbodies of particular type (e.g., lakes, rivers, ponds, streams, wetlands) in a State; a watershed; an individual waterbody; or, a segment of a waterbody. A site may also include intermittent and ephemeral waters that are by nature highly variable on seasonal, annual, and inter-annual time-scales. The nature of this variability will in large part, determine the types of fish that may be encountered in these waterbody types. The appropriateness of a BAF developed for fish that may inhabit these waters is a very site-specific decision, and some aspects of this guidance may not be applicable to such waters. As such, EPA recommends that a state or tribe consult with EPA prior to beginning development of a site-specific BAF for these types of waters. In general, a site is defined according to the interest or need of the agency or interest group, or can be based on the extent of contamination of a waterbody by a bioaccumulative chemical. For example, many site-specific BAFs will be determined at the State level, to support fish consumption advisories issued by the States. Another example would include site-specific BAFs for watersheds in the Total Maximum Daily Load (TMDL) process. Site-specific BAFs may also be determined for waterbodies and waterbody segments receiving point source discharges such as industrial or municipal effluents, combined sewer overflows (CSOs) and stormwater outfalls. Other sites could include depositional areas where contaminated sediments accumulate and bioaccumulation potential is enhanced (i.e., areas where water velocity slows and organic-rich sediments are deposited), or areas where contaminated sediments are disturbed by dredging activities.

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The spatial scale of both BAFs and BSAFs should also be related to the home[5] range of the aquatic organism of interest. With the notable exception of migratory species such as striped bass and some species of eels and salmon, this range will typically be confined to a single waterbody. Even at this scale, however, measuring BAFs (or alternatively BSAFs) may not be the preferred method of determining site-specific BAFs. For example, the difficulty or expense of measuring the concentration of some chemicals in water may be prohibitive. In these situations, it may be desirable to extrapolate a site-specific BAF using a high-quality baseline BAF or BSAF from a comparable site, or from a national BAF based upon a substantial number of measurements. In other cases, a site-specific BAF predicted from the product of a BCF or an octanol-water partition coefficient (K_{ow}) and a food chain multiplier may be preferred. This could be the case when site-specific data are very limited.

For sites larger than a single waterbody, the methods preferred for determining site-specific BAFs may be different than those preferred for a single waterbody. In particular, developing either a field-measured BAF or BSAF (site-specific BAF methods 1 and 2) can become impractical because each waterbody must be sampled, and the necessary sampling effort increases as the number of waterbodies increases. On the other hand, for the other methods of determining site-specific BAFs the sampling effort increases marginally (methods 3 and 4) or not at all (methods 5 and 6) as the number of waterbodies increases.

The investigator should carefully consider trade-offs between the management objective or need (e.g., state, waterbody, area of concern, Superfund site) versus the spatial heterogeneity in bioaccumulation within that site. For large sites, the site-specific BAF must necessarily represent the BAFs for all waterbodies or ecosystems within the site. The within-site variation in BAFs among waterbodies should be minimal to estimate an accurate site-specific BAF for a large site. This requirement can only be met if the waterbodies in the site are comparable in terms of the ecosystem factors known to influence bioaccumulation potential (e.g., chemical loadings histories [sediment-water disequilibrium]; food web structure; organism health and

[5] Depending upon the characteristics of the site, chemical of interest, and target species, as well as the predominant bioaccumulation exposure pathway(s), it may be more appropriate to relate spatial scale of the site to the *foraging range* instead of the *home range*. Although we refer to *home range* throughout this document, the investigator should understand that *foraging range* may be more appropriate depending upon these site-specific factors.

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physiology; water quality factors such as temperature; and food quality). The issue is not simply one of size, but rather the likelihood that the variability in bioaccumulation (and the underlying factors such as sediment-water disequilibria, bioavailability and biomagnification) will increase with the size of the site, and information on these factors should be considered when defining the site. One approach that may improve the comparability of these ecosystem factors for large-scale sites is to derive site-specific BAFs for each type of waterbody (e.g., lakes, rivers, ponds, streams, estuaries, wetlands) within a State, Territory or other region. Even if this approach is

used, it is still important for the investigator to evaluate the comparability of the chemical bioaccumulation potential for the waterbodies within the site.

For large-scale sites, EPA recommends that States, Territories and authorized Tribes consider using the national BAFs, or the baseline BAFs for individual species, to determine the site-specific BAFs. National BAFs, if available, are based upon the highest-quality data for bioaccumulation potential, a careful evaluation of the assumptions made in predictions or estimates, and use a weight-of-evidence determination approach. As a result, the national BAFs are considered reliable estimates of bioaccumulation potential at larger geographic scales. For these reasons, considerable information would be lost if a site-specific BAF were developed without incorporating the national BAF values or the baseline BAFs for individual species that are referenced in the Water Quality Criteria documents for specific chemicals. At large-scale sites, careful determination and justification will be needed as to why bioaccumulation data[6] used for deriving EPA national bioaccumulation factors are not considered applicable to the site.

It is important to identify the fish consumption habits of local populations because the commonly-consumed fish serve as the dietary exposure pathway for bioaccumulative chemicals. EPA encourages States, Territories and authorized Tribes to use local or regional fish consumption data when developing and adopting criteria for their water quality standards, because local or regional fish and shellfish consumption patterns can differ substantially from national consumption patterns. BAFs vary between aquatic species due to several factors, including trophic level, benthic versus pelagic feeding preferences and habitat preferences,

[6] Or, an appropriate *subset* of the bioaccumulation data used to calculate the national BAFs.

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growth rate and migration. Even more variation is possible when one considers the different types of tissues that individuals may consume. Thus, the preferred approach for determining BAFs, as well as many of the details associated with data collection efforts to support their derivation, will depend upon identifying the fish species and tissue types commonly consumed by the local populations. In all cases, the primary selection criterion should be that the target species is among the species commonly consumed in the study area, and that the species is of recreational or sustenance fishing value.

2.5 WHAT ARE THE METHODS FOR DERIVING SITE-SPECIFIC BAFS?

This section provides an overview of the methods for deriving site-specific BAFs. These include:

1. Site-specific BAFs calculated from field data obtained from the site of interest (i.e., "field measured" BAFs);

2. Site-specific BAFs predicted from biota-sediment accumulation factors (BSAFs) calculated from field data obtained from the site of interest;

3. Site-specific BAFs predicted from (3a) extrapolated BSAFs or (3b) bioaccumulation equivalence factors (BEFs) measured at a reference site;

4. Site-specific BAFs predicted from (4a) laboratory-measured BCFs or (4b) the chemical's *n*-octanol-water partition coefficient (K_{ow}), combined with a site-specific food-chain multiplier;

5. Site-specific BAFs recalculated from national or baseline BAFs by adjusting the tissue lipid content and/or organic carbon concentration to reflect site-specific conditions.

The approach to deriving site-specific BAFs using methods 1 and 2 involves measuring new baseline BAF values. Method 3 involves extrapolating measured values from other sites. Methods 4 and 5 involve derivation of site-specific BAFs based on adjustment of existing national or baseline BAFs. In most situations, the first approach (measure new baseline BAF values) is preferable, when resources and data availability permit. We summarize each of the site-specific BAF methods below, and relate each to the corresponding method in the national BAF methodology. As noted in Section 1, the methods for deriving site-specific BAFs are

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closely related to the methods presented in TSD Volume 2 for calculating national BAFs. In Sections 3 through 5 of this TSD, we describe each of the recommended methods in greater detail, emphasizing the scientific basis for each method and technical issues associated with implementing each approach.

2.5.1 Site-specific Field-Measured BAFs

The most direct measure of site-specific bioaccumulation is a BAF derived from data obtained from samples of tissue and water collected from the site of interest, referred to here as a "site-specific field-measured BAF." Because the data are collected from a natural aquatic ecosystem, a field-measured BAF reflects an organism's exposure to a chemical through all relevant exposure routes (e.g., water, sediment, diet). A field-measured BAF also reflects factors that influence the bioavailability, biomagnification and metabolism of a chemical in the aquatic organism or its food web. Therefore, field-measured BAFs are appropriate for all chemicals, regardless of the extent of chemical metabolism in biota from a site. This is site-specific BAF derivation method 1, and it corresponds to Method 1 of EPA's national bioaccumulation methodology.

2.5.2 Site-specific BAFs Predicted from Measured Biota-sediment Accumulation Factors (BSAFs)

The investigator can predict a site-specific BAF from a BSAF that is calculated from the concentrations of a chemical in tissue and sediment samples from the site of interest. The sediment sample must be representative of the surficial sediment within the home range of the organism. A BSAF is similar to a field-measured BAF in that the concentration of a chemical in a biota sample reflects an organism's exposure through all relevant routes. A BSAF also accounts for bioavailability and chemical metabolism in the aquatic organism or its food web. A BSAF may be converted to a BAF based upon the distribution of the chemical between sediment and water, which can be either estimated or measured for a reference chemical. This is site-specific BAF derivation method 2, and it corresponds to Method 2 of EPA's national bioaccumulation methodology. This method is appropriate for moderate to highly hydrophobic nonionic organic

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chemicals, and certain ionic organic chemicals that exhibit lipid and organic carbon partitioning behavior similar to that of nonionic organic chemicals.

2.5.3 Site-specific BAFs Predicted from Extrapolated BSAFs or BEFs Measured at a Reference Site

The investigator may extrapolate site-specific BAFs from BSAFs measured at another (reference) site using two approaches. The first approach is to directly extrapolate a high-quality BSAF (as discussed in Section 4) to the site of interest, if one is available for the chemical of concern. Alternatively, if a high-quality BSAF for a reference chemical is available for the site of interest, then the investigator can use a bioaccumulation equivalence factor (BEF, defined as the ratio between BSAFs for the chemical of concern and the reference chemical) measured at a reference site to extrapolate a BSAF. Since these are actually two related methods, we refer to BSAF extrapolation as method 3a and BEF extrapolation as method 3b. For either method, conversion of the BSAF into a site-specific BAF is accomplished using Method 2 of EPA's national bioaccumulation methodology. Methods 3a and 3b are appropriate for moderate to highly hydrophobic nonionic organic chemicals, and to certain ionic organic chemicals for which similar lipid and organic carbon partitioning behavior applies. Section 5 of this document provides a full description of the BSAFs and BEFs extrapolation methods, and Section 5.1.3 addresses how to choose a reference site.

2.5.4 Site-specific BAFs Predicted from Laboratory-Measured BCFs Combined with a Food Chain Multipliers

The investigators can predict site-specific BAFs as the product of laboratory-measured BCF values and a food chain multiplier (FCM). A laboratory-measured BCF typically reflects only the accumulation of a chemical through the organisms' exposure to water. The BCF will likely underpredict BAFs for chemicals for which accumulation from sediment or dietary sources is important, including hydrophobic nonionic organic chemicals. For such chemicals, a food-chain multiplier (FCM) should be used to adjust the value of a laboratory-measured BCF to better account for chemical accumulation through the food web as a result of dietary exposures. The investigator should measure, estimate (from existing data), or predict (using food chain

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models) the FCM to reflect biomagnification of the chemical for a particular trophic level under site-specific conditions.

A laboratory-measured BCF often reflects the chemical metabolism that occurs in an organism of interest during the BCF measurement. However, a BCF experiment will not account for metabolism of a chemical that occurs at lower trophic levels in the food web because the experiment excludes chemical accumulation from dietary sources. Estimating site-specific BAFs using laboratory-measured BCFs and a food chain multiplier is appropriate for all chemicals, although the investigator should apply this method with caution to chemicals which metabolize in biota, because the method may overpredict BAFs for such chemicals. This is site-specific BAF derivation method 4a, and it corresponds to Method 3 of EPA's national bioaccumulation methodology.

2.5.5 Site-specific BAFs Predicted from *N*-octanol Water Partition Coefficient (K_{ow}) Combined with a Food Chain Multipliers

The investigators can also predict a site-specific BAF for nonionic organic chemicals by using the product of the chemical's K_{ow} and a FCM for a particular trophic level under site-specific conditions. The K_{ow} is strongly correlated with the BCF for this class of chemicals, particularly for those chemicals that are poorly metabolized by aquatic organisms. For these chemicals, the investigator can substitute the measured or predicted K_{ow} for the BCF when predicting a site-specific BAF. The investigator must also adjust the K_{ow} with a FCM to account for chemical accumulation through the food web as a result of dietary exposures, for nonionic organic chemicals where food web exposure is important. This is site-specific BAF derivation method 4b, and it corresponds to Method 4 of EPA's bioaccumulation methodology. This method is appropriate for non- or poorly-metabolized nonionic organic chemicals, but can also be applied to certain ionic chemicals having similar partitioning behavior. This approach may overpredict BAFs for chemicals that are metabolized by aquatic organisms, because metabolism is not incorporated in either the K_{ow} or the FCM.

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2.5.6 Site-specific BAFs Recalculated from National or Baseline BAFs

The investigators can recalculate a site-specific BAF from baseline or national BAFs for a chemical by modifying the default values for the aquatic organism lipid content and/or the dissolved organic carbon (DOC) concentration. The investigator can modify these parameters in the national BAF calculation by:

1. Conducting site-specific field studies to generate representative data,
2. Conducting a literature search to obtain data more representative of local conditions, and/or
3. Selecting an appropriate subset of the national database that EPA used to derive the default values.

Site-specific BAFs recalculated from baseline or national BAFs are appropriate for all chemicals, regardless of the extent of chemical metabolism in biota. This is site-specific BAF derivation method 5, and is an extension of Method 1 of EPA's bioaccumulation methodology.

2.5.7 Advantages and Limitations of Site-specific BAF Approaches

There are method-specific strengths and limitations which the investigator should consider and balance when deriving site-specific BAFs using the methods summarized above. These strengths and limitations, as summarized in Table 2-1, form the basis for selecting approaches to derive site-specific BAFs. Resource limitations, institutional context, and the use to which the BAFs will be put may also be important selection factors. In general, all else equal, measuring new technical baseline BAF values (methods 1 and 2) is preferable to extrapolating or adjusting existing baseline BAFs (methods 3 through 5). For example, the field-measured BAF method is advantageous because it applies to all chemical types, and because it accounts for site-specific factors that affect bioavailability, biomagnification, and metabolism. Nevertheless, field-measured BAFs cannot be readily determined for chemicals that are very difficult to accurately measure at low concentrations in the water column (e.g., 2,3,7,8-TCDD). Site-specific BAFs derived from field-measured BSAFs offer a number of the same strengths as

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field-measured BAFs (e.g., they account for biomagnification, metabolism, and site-specific factors affecting bioavailability). In addition, the BSAF approach is the only field-based method that the investigator can use for chemicals such as 2,3,7,8-TCDD that are difficult to measure in ambient water. However, application of the BSAF method is currently limited to nonionic organic chemicals of moderate to high hydrophobicity. Burkhard et al. (2003a) discuss the relative merits of site-specific BAF versus BSAF measurements for different classes of bioaccumulative chemicals. In general, BSAF approach (Method 2) will be preferable for moderately to highly-hydrophobic organic chemicals, while for less hydrophobic organic chemicals, ionic organic chemicals and inorganic and organometallic chemicals, field-measured BAFs (Method 1) will be the preferred approach. Aside from producing the highest-quality site-specific BAFs, these methods also increase the available bioaccumulation dataset. As noted in Table 2-1, these methods may not be preferred for determining BAFs for large-scale sites (e.g., sites that encompass multiple waterbodies or ecosystems), because the level of effort associated with sampling increases with the number of waterbodies. Further guidance regarding the relative level of confidence associated with each approach is offered in Sections 4.6.1 and 5.2.3.1 and Burkhard et al. (2003b). As more data become available to support derivation of site-specific BAFs by the different methods, it may be possible to generalize the ranges of relative errors or changes in the confidence intervals associated with each method's assumptions, as demonstrated by Arnot and Gobas (2004) for bioaccumulation predictions made with alternative models.

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Table 2-1. Strengths and Limitations of the Methods for Deriving Site-specific BAFs (SS BAFs)

SS BAF Derivation Approach	SS BAF Method	Strengths	Limitations
Derive new baseline BAF values	1. Field measured SS BAF	• Preferred method applicable to all chemical types • Incorporates chemical biomagnification and metabolism • Reflects site-specific attributes that affect bioavailability and dietary exposure	• Representative chemical concentration in water may be difficult to quantify • Level of effort increases with spatial scale, number and type of waterbodies within site
	2. SS BAF predicted from measured BSAF	• Preferred method for highly hydrophobic chemicals • Incorporates chemical biomagnification and metabolism • Reflects site-specific attributes that affect bioavailability and dietary exposure • Useful for chemicals that are difficult to analyze in water • Use of chemical concentrations in sediment reduces temporal variability	• Limited to nonionic organic chemicals with log $K_{ow} \geq 4$ • Accuracy depends on representativeness and quality of the estimate of chemical distribution between sediment and water • Locating representative sediment sampling locations may be difficult • Level of effort increases with spatial scale, number and type of waterbodies within site
Extrapolate measured values from other sites	3. SS BAF extrapolated from BSAF (3a) or BEF (3b)	• Incorporates chemical biomagnification and metabolism • Quality of BSAFs or BEFs measured at another site may be superior to site-specific measurements	• High-quality data currently limited to few sites and chemicals • 3b: Limited to nonionic organic chemicals with log $K_{ow} \geq 4$ • 3b: Accuracy depends on representativeness and quality of the estimate of chemical distribution between sediment and water

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Table 2-1. (continued) Strengths and Limitations of the Methods for Deriving Site-specific BAFs (SS BAFs)

SS BAF Derivation Approach	SS BAF Method	Strengths	Limitations
Adjust existing national or baseline BAFs	4a. SS BAF predicted from BCF and FCM	• Applicable to all chemical types (although FCMs have only been developed for nonionic organic chemicals) • Level of effort does not increase with spatial scale, number and type of waterbodies within site • BCF may account for chemical metabolism in target organisms • Large BCF database available • Standardized test methods	• May not account for chemical metabolism in food web • High-quality data currently limited for highly hydrophobic chemicals • FCM predicted using food chain model is uncertain unless confirmed with site-specific data
	4b. SS BAF predicted from K_{ow} and FCM	• Readily applied with minimal input data • Level of effort does not increase with spatial scale, number and type of waterbodies within site	• Limited to nonionic organic chemicals • Chemical metabolism, when present, not accounted for • Accuracy depends on accuracy of K_{ow} • FCM predicted using food chain model is uncertain unless confirmed with site-specific data
	5. SS BAF recalculated from baseline BAF	• Quality of baseline or National BAFs may be superior to site-specific measurements	• High-quality data currently limited to few sites and chemicals • Depending on method used to derive national BAF, may or may not incorporate chemical biomagnification and metabolism

Extrapolating site-specific BAFs from BSAFs or BEFs measured at a reference site, or recalculating site-specific BAFs from national or baseline BAFs, are methods that the investigator should consider if high quality data are available for the chemical of concern. In such cases, extrapolating or recalculating BAFs may be the most effective way to quantify site-specific bioaccumulation. Unfortunately, high quality data are currently limited to relatively

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few chemicals and sites. The issue of what constitutes "high quality" data for BAFs and BSAFs are discussed in Sections 3 and 4.

Site-specific BAFs predicted using a BCF or K_{ow} and food chain multiplier have the advantage of requiring limited site-specific data, and can be readily applied to many sites, or sites that encompass many waterbodies. BAFs predicted from a laboratory-measured BCF and a FCM can be applied to all chemical types, and data for BCFs are generally more plentiful than data for field-measured BAFs. However, acceptable BCFs for highly hydrophobic chemicals (i.e., those with a log $K_{ow} > 6$) appear to be very limited, often because of lack of ancillary data that affect bioavailability (e.g., dissolved organic carbon). Deriving site-specific BAFs using K_{ow} and FCMs (where appropriate) offers a distinct advantage in that no laboratory data (besides a K_{ow}) or field data are needed to derive a BAF. However, this method is limited to nonionic organic chemicals that are non- or poorly-metabolized. Finally, if the FCMs used in either of these approaches is predicted with a food chain model, then the accuracy of the FCM may be questionable unless the prediction is confirmed by data. Burkhard et al. (2003b) compared the performance of predictions made using national bioaccumulation methodologies 2 and 4, and found that method 4 was more sensitive to ecosystem conditions, particularly the temporal dynamics of several important factors (lipid, foodweb structure, and exposure concentrations). TSD Volume 2 (USEPA, 2003) and a number of other publications (Burkhard et al. 2003a and 2003b) provide further discussions of the advantages and limitations of the site-specific BAF approaches, and the possible trade-offs between different methods.

2.5.8 Weight-of-Evidence Approach to Selecting a Site-specific BAF

The final site-specific BAF must be selected from the individual BAFs by using a weight-of-evidence approach that takes into account the uncertainty in the individual BAFs and the data preference hierarchy (i.e., field-measured BAFs are preferred over BAFs derived using the other methods). Investigators are encouraged to determine site specific BAFs using all of the possible methods available. As noted in the previous sections, selecting the most appropriate derivation procedure depends greatly on chemical properties. Section 5.4.2 of the 2000 Human Health Methodology provides a guide for selecting the most appropriate final BAF when the uncertainty

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is similar between two individual baseline BAFs calculated using different methods. Section 6.1 of TSD Volume 2 and Section 5.4.3.2 of the 2000 Human Health Methodology provide more detailed discussions of this step.

All BAF values should be reviewed carefully to assess their sufficiency, quality, variability, and overall uncertainty. Large differences in individual site-specific BAFs for a given species or trophic level (e.g., greater than a factor of 10) should be investigated further. As a result, some or all of the site-specific BAFs for a given trophic level might not be used. Procedural and quality assurance guidelines are described in Sections 3 and 4, and should be used to evaluate the quality, variability, and uncertainty of site-specific BAFs.

The data preference hierarchy for each BAF derivation procedure (Figure 2-1 and further detailed in Table 2-1) is based on the relative strengths and limitations of each BAF method and reflects the general preference of field-measured data over laboratory- or model-based estimates of bioaccumulation. Importantly, this hierarchy is intended for use as a guide for selecting the final baseline BAF rather than as a steadfast rule. Departures from this data preference hierarchy are entirely appropriate when considerations of uncertainty and weight of evidence indicate that a lower tier method would be preferred over a higher tier method. In general, when site-specific BAFs are available for more than one BAF method within a given trophic level, the final site-specific BAF for each trophic level should be selected from the most preferred BAF method. If uncertainty in a trophic level–mean baseline BAF based on a higher tier (more preferred) method is judged to be substantially greater than one from a lower tier method, and the weight of evidence from the various methods suggests that a BAF value from a lower tier method is likely to be more accurate, then the final baseline BAF for that trophic level should be selected from the lower tier method.

When the weight of evidence among the various BAF methods is being considered, greater confidence in a site-specific BAF is generally assumed when the BAFs are in agreement across a greater number of methods within a given trophic level. However, lack of agreement among site-specific BAFs derived from different methods does not necessarily indicate less confidence, if such disagreements can be adequately explained. For example, if the chemical of

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concern is metabolized by aquatic organisms represented by a baseline BAF value, one would expect disagreement between a baseline BAF derived from a field-measured BAF (the highest priority data) and a baseline BAF predicted from a K_{ow} and model-derived FCM. In addition, consideration should also be given to the quantity and diversity of bioaccumulation measurements that underlie the calculation of a trophic level–mean baseline BAF. In some cases, the uncertainty associated with very limited BAF data from a "more preferred" method may be offset by the greater quantity and diversity of data that are available from an otherwise "less preferred" method for a given data preference hierarchy.

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ED_006490_00009970-00056

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3. MEASURING SITE-SPECIFIC BIOACCUMULATION FACTORS

Bioaccumulation factors are used to relate chemical concentrations in aquatic organisms to concentrations in the ambient media (e.g., water and sediment) of aquatic ecosystems. The most direct measure of site-specific bioaccumulation is a BAF derived from data obtained from samples of tissue and water collected from the site of interest. These data are then used to calculate a site-specific, field-measured BAF. A field-measured BAF reflects an organism's exposure to a chemical through all relevant exposure routes (e.g., water, sediment, diet), because the data are collected from a natural aquatic ecosystem. A field-measured BAF also reflects factors that influence the bioavailability, biomagnification and metabolism of a chemical in the aquatic organism and/or its food web. Therefore, field-measured BAFs are appropriate for all chemicals, regardless of the extent to which these factors influence bioaccumulation at the site.

Two forms of the BAF are used by EPA in the 2000 Human Health Methodology (USEPA, 2003). The first is the total BAF, denoted $BAF^{t}_{i,T}$, also referred to as the "field-measured" BAF. The $BAF^{t}_{i,T}$ is calculated from the total concentration of chemical in the appropriate wet tissue of the aquatic organism sampled at trophic level *i,* and the total concentration of the chemical in the ambient water at the sampling site:

$$\text{Total BAF} = BAF^{t}_{i,T} = \frac{C_t}{C_w} \qquad \text{(Equation 3-1)}$$

where:

C_t = total concentration of the chemical in tissue
C_w = total concentration of chemical in water

Average or mean chemical concentrations are used for each phase in the calculation of the total BAF (Equation 1), since multiple samples of biota and water should be collected to characterize chemical concentrations at a site. Calculating a total BAF is presented in the following example.

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Calculation of site-specific Total BAFs from measurements at the site (Method 1)

A hypothetical lake is contaminated by chemical *x*. Data obtained from field studies in the lake indicate that the mean concentration of the chemical in the water column, 49.5 µg/L, reflects adequate temporal and spatial averaging, based on the K_{ow} of this chemical. Consumption surveys of the local population indicate that crayfish (*Orconectes sp.*) is a commonly consumed organism, and was selected as a target organism for sampling and BAF determination. Review of the trophic level assignment of aquatic species corresponding to CSFII consumption categories (Table 6-4 in TSD Volume 2 [USEPA, 2003]) indicates that crayfish that are commonly consumed by the general U.S. population belong to trophic level 2. Based on the field studies, the average chemical concentration in crayfish is 2.4 mg/kg. Data obtained from field studies also indicates that the mean concentration of the chemical in the water column is representative of the average exposure of chemical *x* to the crayfish. The total BAF is calculated using equation 3-1:

$$BAF_{i,T}^{t} = \frac{C_t}{C_w} \qquad \text{(Equation 3-1)}$$

$$BAF_{2,T}^{t} = \frac{C_t}{C_w} = \frac{2.4\ \text{mg}}{\text{kg}} \cdot \frac{\text{L}}{49.5\ \mu\text{g}} \cdot \frac{1000\ \mu\text{g}}{mg} = 48.5\ \text{L/kg}$$

The site-specific total BAF for chemical *x* in crayfish is 48.5 L/kg. Generally, site-specific total BAFs would also be determined for commonly consumed organisms from trophic levels 3 and 4.

The second form of bioaccumulation factor is the baseline BAF, which is applied specifically to nonionic organic chemicals. The baseline BAF ($BAF_{i,L}^{fd}$) is calculated using the lipid-normalized concentration in tissue and the freely dissolved chemical concentration in the water:

$$\text{Baseline } BAF_i = BAF_{i,L}^{fd} = \frac{C_l}{C_w^{fd}} - \frac{1}{f_l} \qquad \text{(Equation 3-2)}$$

where:

C_l	=	lipid-normalized concentration of the chemical in tissue
C_w^{fd}	=	concentration of chemical that is freely dissolved in water
f_l	=	mass fraction of wet tissue that is lipid

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Again, average or mean chemical concentrations are used for each phase in Equation 3-2.

The baseline BAF is also related to (but not the same as) the lipid-normalized and freely dissolved-based bioaccumulation factor ($BAF_{i,l}^{fd}$):

$$\text{Baseline } BAF_i = BAF_{i,l}^{fd} - \frac{1}{f_l} \qquad \text{(Equation 3-3)}$$

The derivation of the baseline BAF and its relationship to $BAF_{i,l}^{fd}$ (Equation 3-3) is discussed in TSD Volume 2 (USEPA, 2003) and Arnot and Gobas (2004). Calculating a baseline BAFs is presented in the following example.

Calculation of site-specific Baseline BAFs from measurements at the site (Method 1)

This example illustrates the development of a site specific, trophic level 4 BAF using Method 1 for a nonionic, hydrophobic organic chemical (chemical *x*). Because this is an organic chemical, the site-specific BAF should be calculated as a baseline BAF from measurements of lipid-normalized chemical concentrations in consumed tissue and freely-dissolved concentrations in ambient water at the site. Calculating a baseline BAF facilitates comparison to other BAF values and may reduce the variance of the BAF. The baseline BAF can be converted to a total BAF for calculation of a water quality standard for the site.

Site-Specific Data

A field study was conducted in an unnamed river to measure concentrations of chemical *x* in the aquatic food chain and the water column, to support the development of site-specific BAFs. A review of the dietary preferences of the sport fish caught and consumed by the local population indicated that largemouth bass was a preferred species at trophic level 4. Therefore, this fish was targeted for collection during sampling in 1993, and three composite samples were analyzed. Twelve water samples were also collected on a near-monthly basis in 1993. As recommended in the Section 3 guidance, lipid contents were measured in all fish composite samples, and dissolved and particulate organic carbon (DOC and POC) concentrations were measured in all water samples. The following data were obtained from the study:

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Calculation of site-specific Baseline BAFs from measurements at the site (continued)

LARGEMOUTH BASS		
Date	**Chemical *x* Concentration (µg/g)**	**% Lipid**
August-93	0.797	1.16
August-93	1.040	1.45
August-93	0.646	1.10

WATER COLUMN			
Date	**Chemical *x* Concentration (ng/L)**	**DOC Concentration (mg/L)**	**POC Concentration (mg/L)**
January-93	1.02	5.18	1.49
February-93	1.01	5.47	1.55
March-93	1.43	5.53	1.98
April-93	2.34	4.39	0.36
May-93	2.24	4.41	0.37
May-93	2.47	5.12	1.46
June-93	3.32	4.84	1.08
June-93	3.74	5.32	1.55
July-93	4.00	5.53	2.31
August-93	2.92	4.83	0.37
August-93	2.89	4.95	1.46
September-93	2.26	4.90	1.38

Review of these data indicated that the mean concentration of the chemical in the water column reflects adequate temporal and spatial averaging, based on the hydrophobicity of this chemical ($\log K_{ow} = 5.84$), and was representative of the average exposure of chemical *x* to the target fish.

Lipid and Freely-dissolved Normalization of Concentration Data

Chemical concentrations in fish (C_t) were normalized by the lipid content (f_l) of each sample:

$$C_l = C_t / f_l$$

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Calculation of site-specific Baseline BAFs from measurements at the site (continued)

The lipid-normalized chemical concentration (C_l) in each sample is tabulated:

Sampling Date	Lipid-normalized Concentration (µg/g-lipid)
August-93 (1)	68.7
August-93 (2)	71.7
August-93 (3)	58.7
August sample average	66.4

The lipid-normalized chemical concentrations in largemouth bass were then averaged, to determine a mean value of 66.4 µg/g-lipid.

The freely dissolved fraction of chemical in the water column (f_{fd}) was also calculated for each sample, using equation 3-6:

$$f_{fd} = 1 / (1 + POC \cdot K_{ow} + 0.08 \cdot DOC \cdot K_{ow}) \qquad \text{(Equation 3-6)}$$

For example, the freely dissolved chemical fraction of the January water sample is:

$$f_{fd} = \frac{1}{1 + \frac{1.49mg - POC}{L} \cdot 10^{5.84} \frac{L}{kg} \cdot \frac{kg}{10^6 mg} + 0.08 \cdot \frac{5.18mg - DOC}{L} \cdot 10^{5.84} \frac{L}{kg} \cdot \frac{kg}{10^6 mg}}$$

$$= 0.431$$

The freely dissolved chemical concentration (C_w^{fd}) is calculated as:

$$C_w^{fd} = f_{fd} \cdot C_w$$

The freely dissolved fraction and freely dissolved chemical concentration is calculated for each sample as tabulated below:

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Calculation of site-specific Baseline BAFs from measurements at the site (continued)

Sampling Date	f_{fd}	Freely-dissolved Concentration (ng/L)
January-93	0.431	0.440
February-93	0.421	0.425
March-93	0.374	0.534
April-93	0.670	1.57
May-93	0.667	1.49
May-93	0.436	1.08
June-93	0.496	1.65
June-93	0.423	1.58
July-93	0.344	1.38
August-93	0.656	1.92
August-93	0.438	1.27
September-93	0.449	1.02

The freely-dissolved chemical concentrations were then averaged, to determine a mean value of C_w^{fd} = 1.20 ng/L.

Calculating a Site-specific Baseline BAF

The site-specific baseline BAF was then calculated using the average C_l (66.4 µg/g-lipid), f_l (1.24%) and C_w^{fd} (1.20 ng/L) as shown below:

$$\text{Baseline BAF}_i = \frac{C_l}{C_w^{fd}} - \frac{1}{f_l} \qquad \text{(Equation 3-2)}$$

$$\text{Baseline BAF}_4 = \frac{66.4\mu g}{g-lipid} \cdot \frac{L}{1.20ng} \cdot \frac{1000ng}{1\mu g} \cdot \frac{1000g}{kg} - \frac{1}{0.0124} = 5.55 \times 10^7 \, L/kg-lipid$$

The site-specific baseline BAF for chemical *x* in largemouth bass is 5.55 $\times 10^7$ L/kg-lipid.

Calculation of site-specific Baseline BAFs from measurements at the site (continued)

Calculating a Site-specific Total BAF

In order to determine a water quality standard for chemical *x* at the unnamed river site, the site-specific baseline BAF must be converted to a total BAF. Recalling the relationship between the baseline BAF and the total BAF ($BAF^{t}_{i,T}$):

$$\text{Site Specific } BAF^{t}_{i,T} = (f_l \cdot \text{Baseline } BAF_i + 1) \cdot f_{fd} \qquad \text{(rearranged Equation 3-4)}$$

Using averages of measured values for lipid content (1.24%) and calculated freely dissolved fractions (0.484), the site-specific total BAF can be calculated:

$$\text{Site-Specific } BAF^{t}_{4,T} = \left(0.0124 \cdot 5.55 \times 10^{7} \tfrac{L}{kg-l} + 1\right) \cdot 0.484 = 3.32 \times 10^{5} \tfrac{L}{kg}$$

The site-specific total BAF for chemical *x* in largemouth bass is 3.32×10^{5} L/kg.

There are important advantages to calculating bioaccumulation factors for hydrophobic nonionic organic chemicals as baseline BAFs because these expressions acknowledge the thermodynamic relationships (or fugacities, that can be thought of as chemical pressures) that govern the bioavailability and bioaccumulation of these chemicals and facilitate comparisons across ecosystems (Mackay, 2001). The lipid and freely-dissolved normalizing of concentrations also reduces the variance in BAFs among sites and trophic levels for these chemicals. Lipid normalization is useful for hydrophobic nonionic organic chemicals, because these chemicals partition extensively into the lipid fraction of tissues. For other classes of chemicals, lipid partitioning is usually much more limited, and lipid normalization is not appropriate. Likewise, normalizing the concentrations of hydrophobic nonionic organic chemicals in water by the freely-dissolved fraction is helpful in reducing the variability of BAFs, since only the freely-dissolved phase of the chemical is considered to be bioavailable in water. Hydrophobic nonionic organic chemicals in water are present in the freely dissolved form as well as in association with dissolved or colloidal organic carbon (i.e., commonly measured as dissolved organic carbon) and particulate organic carbon. The freely dissolved chemical is generally only a fraction of the analytically determined concentration, particularly for highly hydrophobic chemicals (log K_{ow} >5.5). Determining the freely dissolved fraction of a nonionic organic chemical in water, by measurement or calculation, is discussed in Section 3.4.2.

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The baseline BAF can be calculated from a BAF_T^t as shown in Equation 3-4 by using information on the lipid fraction (f_l) of the tissue of concern for the study organism and the fraction of the total chemical that is freely dissolved in the ambient water (f_{fd}):

$$\text{Baseline BAF}_i = \left[\frac{BAF_{i,T}^t}{f_{fd}} - 1 \right] \cdot \frac{1}{f_l} \qquad \text{(Equation 3-4)}$$

where:

$BAF_{i,T}^t$ = Total BAF

f_{fd} = fraction of the total concentration of chemical in water that is freely dissolved

TSD Volume 2 (USEPA, 2003) provides more detailed information on derivation of the baseline BAF equation. An alternative formula for the relationship between the total BAF and the baseline BAF is offered by Arnot and Gobas (2004). The latter may be advantageous for calculating baseline BAFs for less hydrophobic organic chemicals that have total BAFs approaching 1.0.

This TSD specifically addresses the determination of site-specific BAFs for nonionic organic chemicals, which generally follow a hydrophobic organic chemical paradigm (i.e., chemicals that preferentially partition into the lipid and organic carbon phases). The investigator should also be aware that not all classes of organic chemicals necessarily follow this paradigm. Examples of "other" classes of organic chemicals, for which this TSD may not apply, include:

- Perfluorinated substances, especially polyfluorinated octyl carboxylic acid (PFOA) and sulfonic acid (PFOS) (Scott et al., 2006; Moody et al., 2002; Giesy and Kannan, 2001),
- Surfactants (Tolls and Sijm, 2000; Tolls et al., 1994);
- Synthetic Dyes & Pigments (Lynch, 2000). Most pigments and many dyes are so sparingly soluble in water that K_{ow} can not be measured.
- Organosilicon compounds (Allen et al., 1997; Fackler et al., 1995). These substances can be sparingly soluble in water and highly volatile thus bioaccumulation testing is difficult.

ED_006490_00009970-00066

- Methylmercury, the highly bioaccumulative form of mercury, is an ionic organic chemical. The methods described in this TSD for determining site-specific BAFs do not apply to methylmercury.

Several of these are ionic organic chemicals; derivation of BAFs for these chemicals is discussed in Section 2.2. BAFs for inorganic/organometallic chemicals is discussed in Section 2.1.

The investigator should be careful to use sensitive analytical methods and appropriate statistical treatment of low-end censored data. Concentrations of bioaccumulative chemicals (especially dissolved concentrations in water) are frequently near or below the analytical detection limit. Where the chemical is present at concentrations below the minimum detection limit (MDL) for the analytical method, the uncensored value should be used in the calculation of the mean concentration. When the chemical is not detected at all (i.e., no response above instrumental noise), ½ of the MDL is commonly used as a replacement value (e.g., in USEPA's Superfund program). However, calculation of BAFs using half of the MDL for concentrations in water can result in spurious and non-predictive BAFs. When chemical concentrations are not detected in some samples, EPA recommends that the investigator apply statistical approaches for averaging with censored data. These include Helsel (2005), Helsel and Hirsch (2005), El-Shaarawi and Dolan (1989), Newman et al. (1989) and Newman (1995). These approaches can be used with normally and log-normally distributed data. Berthouex and Brown (1994) recommend that unbiased means only be calculated from concentration data if fewer than 20% of the reported values are nondetects. The investigator should be aware that even if the methods mentioned above are used, working with low-end censored data introduces greater uncertainty in values both of mean chemical concentrations and of BAFs. Graphical analysis of chemical concentrations in biota and BAFs versus chemical concentrations in water can help the investigator determine whether to include or exclude data for concentrations less than the MDL and/or not detected at all.

Ideally, data obtained from the open literature (e.g., peer-reviewed journals, scientific reports, professional society proceedings) can be used to calculate site-specific BAFs, provided

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that the appropriate measurements have been made and information is available indicating the quality and usability of the data. A number of bioaccumulation databases compile such information. Weisbrod et al. (2007) provide a review of databases containing information for BCFs, BAFs and BSAFs. At the present time, the BAF database of Arnot and Gobas (2006)[1] is recommended as a resource to investigators. This database contains 1,656 BAF values measured for 842 organic chemicals in 219 aquatic species. What makes this database especially useful is that it includes a data quality assessment according to 6 criteria and rates each BAF measurement with an overall confidence level. This data quality assessment is valuable as guidance for investigators, who should ensure that high quality data are being used to derive BAFs. Another database is the Japanese National Institute of Technology and Evaluation (NITE) Biodegradation and Bioconcentration Database of Existing Chemical Substances: (http://www.safe.nite.go.jp/english/kizon/KIZON_start_hazkizon.html).

Additional bioconcentration and bioaccumulation databases are under development; for example the European Chemical Industry Council (CEFIC) Long Range Research Initiative for Predicting the Environmental Fate of Chemicals. Once published, these and other databases may also become valuable resources to the investigator. Given sufficient supporting information, the investigator could calculate reliable site-specific BAFs and make some assessment of the overall uncertainty in the BAF values. Unfortunately, relatively few high-quality bioaccumulation datasets are available, and those that do exist are limited in terms of number of chemicals, sites and species of interest. Therefore, it will generally be necessary for the investigator to generate the data required to derive the BAF by sampling at the specific site.

EPA prefers to use field-measured BAFs when developing water quality standards for the protection of human health (USEPA, 2000a). However, protocols for measuring site-specific BAFs have not previously been available. Although a field-measured BAF is a direct measure of bioaccumulation at a site, the BAF will only have predictive power if a number of important factors are properly addressed in the design of the field sampling effort. This Section provides guidance to the investigator considering this method of deriving site-specific BAFs and

[1] The Arnot and Gobas BCF/BAF database is available at http://pubs.nrc-cnrc.gc.ca/cgi-bin/rp/rp2_supp_e?er_a06-005_er4-06

ED_006490_00009970-00068

specifically addresses the design of plans for the collection of biota and water samples necessary to determine accurate BAF values.

In the next section (3.1), a series of key questions are presented to the investigator faced with designing a field study to determine a site-specific BAF. Section 3.2 illustrates several methods to assess the variability of site-specific BAF prior to sampling and to develop a field study design based upon this variability. Following that are sections that address sampling design considerations necessary to measure site-specific BAFs specific to biota (Section 3.3) and water (Section 3.4).

3.1 KEY STUDY DESIGN QUESTIONS FOR DETERMINING SITE-SPECIFIC BIOACCUMULATION FACTORS

Although a field-measured BAF is a direct measure of bioaccumulation at a site, this does not mean that it is simple to collect the data necessary to determine an accurate BAF value. In fact, of all the methods presented in this TSD for determining site-specific BAFs, collecting field data at the site of interest is probably the most difficult approach. The text box below highlights several key factors for the investigator to consider for a field study. The most important aspect of conducting a successful field study to measure site-specific BAFs is collecting representative samples of the biota and water. In general, samples will be most representative when the measured concentrations are reflective of long term average concentrations for the chemical in biota and exposure media. The home range of the target species will dictate the spatial scale of the sampling effort. Chemical temporal and spatial distributions, organism life history, and duration of exposure, among other factors, all contribute to BAF uncertainty and should be addressed by the field sampling plan. Investigators are encouraged to report the uncertainty associated with measured site-specific BAFs.

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Key Factors to Consider When Designing a Field Study to Determine a Site-specific BAF

Chemical of Concern:

- Type of chemical (i.e., nonionic organic, ionic organic, inorganic, and organometallic)
- Hydrophobicity
- Metabolism
- Distribution between sediment and water
- Availability of accepted analytical methods
- Sensitivity of analytical method (especially in water)

Target Biota Species:

- Consumption by human population
- Size of consumed organisms
- Trophic level and prey items
- Lipid content
- Migration and movement

The Site:

- Size of site / number of waterbodies
- Sampling characteristics (temporal and spatial variability of chemical concentrations in biota and water)
- Ecosystem type

Sampling requirements for biota and water will largely be controlled by the variability of chemical concentrations at the field site. Chemical concentrations in biota and water vary, both in space and time, in different ways and due to a variety of factors, as discussed in Sections 3.3 and 3.4. As will be seen, the properties of the chemical itself play an important role in defining this variability. Separate field designs and approaches should be considered for sampling water and biota, due to differences inherent in these media. Concurrent sampling of biota and water may not be the optimum field sampling design for many bioaccumulative chemicals. The investigator must take all of these factors into account when specifying a sampling design to determine a field-measured BAF. Additionally, the application of data quality assurance procedures when measuring, estimating, and applying BAFs is very important.

The investigator faced with designing a field study to determine a site-specific BAF should consider the following series of key questions, intended to identify factors of the problem to be addressed by the sampling plan.

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Key Study Design Questions

1. Site Definition. *Have I adequately defined my site of interest in terms of spatial extent?*

- The home range of the target species will dictate the spatial scale of the sampling effort.
- Size of site / number of waterbodies
- Sampling characteristics (temporal and spatial variability of chemical concentrations in biota and water)
- Ecosystem type

2. Study Feasibility. *Can I adequately detect the chemical in water with available analytical methods (e.g., with a detection frequency > 80%)?*

- Investigate detection limits of available analytical methods
- Compare to expected chemical concentrations

3. Precision Goal. What is the minimum level of accuracy in BAF measurements I am willing to accept (i.e., confidence limits within a factor of 2, 5 or 10)? How do I determine the level of effort (i.e., the number and type of biota and water samples) associated with different levels of accuracy?

- Data Quality Objectives (DQO) process (USEPA, 2000c)
- Bootstrap or Monte Carlo simulations
- Bioaccumulation modeling

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4. Biota. *Which species should I sample?*

- Consider consumption patterns of human population
- Availability of species of appropriate size at the site
- Diversity of exposure pathways (i.e., benthic & pelagic)
- Dietary composition/trophic status

5. Temporal Variability (i.e., Sampling Event Frequency). *How many times do I need to sample biota and water at the site?*

Biota Sampling Considerations

- Consider chemical properties (hydrophobicity and metabolism)
- Consider biota characteristics (migration, reproduction, availability, other seasonal characteristics based on climate, etc.)
- Consider consumption pattern (e.g., times of year they are harvested)
- Time of year and temperature, as related to the dynamics of lipid content
- Lessons learned from bootstrap/simulation examples

Water Sampling Considerations

- Consider chemical properties (hydrophobicity)
- Consider ecosystem conditions (e.g., variability due to hydrodynamics) and temporal aspects of chemical loadings
- Lessons learned from bootstrap/simulation examples

6. Spatial Variability (i.e., Number of Stations). *How many locations should be sampled?*

- Consider evidence of spatial gradients in exposure concentrations as well as the presence of sources
- Biota characteristics (mobility/home range, habitat preference, etc.)
- Consumption characteristics (harvesting areas)
- Ecosystem properties (size of site, spatial differences in hydrodynamics, etc.)
- Consider spatial sampling design options (e.g., random, stratified, systematic, judgment)

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7. Biota Sample Type. What types of biota samples should I collect (i.e., age/size, tissue, quantity, etc)? What ages/sizes of these species are consumed?

- Which tissues are most commonly consumed and how are they prepared?
- Does this vary with organism size?
- Composite vs. individual samples
- Chemical analysis requirements

8. Water Sample Type. *What types of water samples should I collect?*

- Individual grab samples vs. composites?
- Temporal averaging
- Depth integration
- Composite vs. individual samples
- Chemical analysis requirements

9. Chemical Analytical Methods. Which analytical methods should I use?

- Must be specific for the individual chemical(s) of concern

10. Biota Sampling Methods. *How should biota be sampled?*

- Appropriate methods depend on waterbody and target organisms

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11. Water Sampling Methods. *How should water be sampled?*

- Appropriate methods depend on contaminant and waterbody
- Measure total or filtered chemical concentrations?
- "Clean" sampling techniques for different types of chemicals must be used
- Collection and analysis of blank samples

12. Water/Biota Sampling Correspondence. *How should I coordinate biota and water sampling (e.g., concurrent vs. staggered sampling)?*

- Consider chemical properties (hydrophobicity and metabolism)
- Consider ecosystem conditions (variability due to hydrodynamics) and temporal aspects of chemical loadings
- Lessons learned from bioaccumulation model simulations

13. Ancillary Measurements. *What other chemical, water and biota parameters should I consider measuring?*

- For organic chemicals, lipid and dissolved organic carbon are important ancillary measurements
- Useful ancillary measurements for biota include age, sex, trophic status and tagging information
- Total suspended solids (TSS) and particulate organic carbon (POC) are useful ancillary measurements for water
- Chemical concentrations in sediment and sediment organic carbon content are important data
- pH and alkalinity are important measurements for ionic organic chemicals
- Location information, such as GPS coordinates

Of course, the bioaccumulation field data should be collected at the specific site for which criteria are to be applied and with the target species of concern. For large-scale sites, EPA recommends that samples be collected from each waterbody or ecosystem within the site for which site-specific BAFs are to be derived. Sampling in a subset of waterbodies is a valid

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approach only if it can be demonstrated that the resulting BAFs are representative and can be extrapolated to other locations in the site where the criteria and values will be applied. In practice, this may be difficult unless considerable information is available for the waterbodies that comprise the site.

3.2 HOW TO DESIGN A SAMPLING PLAN TO MEASURE BAFS

In designing a field study to measure BAFs, the investigator should determine the appropriate number of biota and water samples to collect as well as their spatial and temporal allocation. Straightforward guidance on this issue is not readily available because study designs vary depending on the temporal and spatial variability of the chemical in the ecosystem and on the dynamics of the chemical in the biota. A successful sampling design procedure for any field study should consider the following ecosystem conditions and chemical properties: spatial and temporal gradients in chemical concentrations; chemical distribution between the sediment and the water column; life history patterns of the organism (e.g., migration, diet, and food web structure and composition); the chemical's hydrophobicity; and metabolism of the chemical in all organisms composing the food web. The investigator defines the frequency of sample collection (i.e., the number and spacing of sampling events in time), the spatial distribution of sample collection locations, and the total number of samples to be collected. Having the appropriate sampling frequency and spatial distribution (the sampling design structure) enables the determination of BAFs that are representative of the long-term average conditions in an ecosystem and provides BAFs with low bias and good accuracy. Lack of bias in the BAF determination depends upon the representativeness of samples collected and analyzed, while precision depends upon the numbers of samples. In the optimization process, precision of the measurements is balanced against the costs associated with sample collection and analysis, and in many cases, compositing of samples is required to limit costs associated with the chemical analyses. These sampling design issues are addressed in this section.

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Three sampling design approaches will be demonstrated in the following subsections (Sections 3.2.1 thru 3.2.3). These approaches are complementary, and the investigator can apply them individually or together to design a sampling plan appropriate for measuring a site-specific BAF.

Section 3.2.1 presents and demonstrates a statistical method (Bootstrap resampling) to determine the required numbers of samples of biota and water necessary to achieve a desired precision for the BAF measurement. This approach uses field data to estimate the relationship between BAF precision and the number of samples collected for chemical analysis in biota and water. Adequate field data sets for this type of evaluation are limited because this analysis requires coordinated fish, water, and sediment data over time. In most field studies, fish and sediment samples might be collected once in a field season because of the labor required for their collection. EPA is aware of relatively few field data sets that are adequate for this type of evaluation (see Section 5.1.1 of TSD Volume 2 [USEPA, 2003] for discussion). These include PCB congener data from the Hudson River (TAMS, 1998), the Green Bay Mass Balance Study (USEPA, 1989b), and the Lake Michigan Mass Balance (USEPA, 2004).

A second approach modifies the Bootstrap method by substituting simulated data generated using Monte Carlo methods for measurements of chemical concentrations in biota and water. The Monte Carlo approach requires less site-specific data than does the Bootstrap procedure, but still gives the investigator a way to estimate to what extent the precision of the BAF depends upon the number of chemical concentration measurements in biota and water. The Monte Carlo approach is presented in Section 3.2.1.2. Monte Carlo results (BAF confidence limits) were compared to results from the Bootstrap, and were also repeated using different variances in chemical concentrations and different degrees of correlation between biota and water chemical concentrations.

A third approach to sampling study design emphasizes the sampling structure (number of sampling events over time and space), using model simulations to evaluate the relative influences of the underlying factors in obtaining representative samples for the BAF determinations. With model simulations, biota and water data can be created on a day-to-day basis assuming different

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ecosystem conditions and chemical properties (e.g., temporal and spatial concentration profiles, life history scenarios, metabolism rate, and hydrophobicity). For these simulations to be meaningful, the model constructs should provide reasonable representations of ecosystem conditions and chemical properties. Model simulations are used to evaluate how the following factors influence the spatial distribution of samples, and the number and temporal spacing of sampling events, required to accurately determine BAFs:

- how the temporal and spatial variability in chemical concentrations,
- the chemical's hydrophobicity and metabolism rate in fish,
- the structure of the aquatic food web (benthic vs. pelagic components), and
- the disequilibrium between chemical concentrations in the sediment and water column.

Section 3.2.2 discusses the sampling design approach using model simulations.

3.2.1 Determining the Number of Samples to Collect

As discussed in the previous section, the precision of a BAF measurement is defined largely by the total number of samples that are collected and analyzed. The investigator designing the sampling plan should determine the necessary numbers of samples based upon the expected variability in chemical concentrations and the goal for precision of the BAF measurement. The investigator should address two problems in order to determine this relationship:

- The variability of the chemical concentration in biota and water should be known or estimated. This is a problem due to the scarcity of high-quality bioaccumulation datasets, and because prior knowledge of chemical concentrations at the site of interest is unlikely.
- Because BAFs are ratios of random variables, no formulas are available for their exact sampling variances (CDM, 2002). It is fairly unusual to collect environmental data specifically for the purpose of calculating a concentration ratio, such as a BAF.

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As a consequence, relatively little attention has been paid to the relationship between the accuracy of a ratio of two random variables and the sample sizes[2].

3.2.1.1 Bootstrap BAF Resampling

The precision of BAF measurements calculated from different numbers of chemical concentration measurements can be estimated by Bootstrap resampling, if data are available. This numerical simulation method can be used by the investigator to determine the required numbers of biota and water samples, once the goal for BAF precision has been established. The Bootstrap method is demonstrated by examples using Green Bay Mass Balance Study PCB congener data in Appendix 3A. The ratio of the 90th to the 10th percentile values of the distribution of BAFs in each bootstrap resampling calculation was found to be a useful measure of variability. This ratio (the confidence limit ratio or CLR) is a measure of the range or width of the BAF confidence interval; a smaller CLR indicates less uncertainty (and greater precision) in the BAF calculated from a given sampling design. The Bootstrap resampling examples show that the precision (as well as the bias) of BAF estimates are sensitive to the sample sizes of chemical concentrations in both fish and water, especially for sample sizes smaller than 6. These examples also indicate that resampling different combinations of the number of fish and water concentrations can yield comparable BAF precision. For example, the same confidence limit ratio for PCB congener 149 forage fish BAF was obtained by resampling 10 fish and 6 water concentrations or 4 fish and 10 water concentrations.

If site-specific data for concentrations of the target chemical in biota and water are available, then Bootstrap resampling is probably the best way to determine the number of samples to collect and analyze in order to determine a BAF of the desired precision. Bootstrap resampling can also be useful if a site-specific BAF is measured and the uncertainty is found to be unacceptably large. In this case, the bootstrap can be used to estimate the additional sampling effort, in terms of numbers of biota and/or water samples, required to improve the precision of

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[2] It should be noted that for individual random variables, such as chemical concentrations in biota or water, there is considerable guidance available regarding the relationship between sample size and the resulting variances. For example, the *DQO-PRO* software program (http://www.instantref.com/download-dqo-pro.html) calculates numbers of samples relative to uncertainties in environmental data. EPA also distributes the program *ProUCL* (www.epa.gov/nerlesd1/tsc/software.htm) which calculates upper bound confidence limits for data using a number of different parametric and nonparametric statistical methods.

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the BAF derived from these measurements. Bootstrap resampling is a much less useful tool when site-specific data for concentrations of the target chemical in biota and water are not available. If the investigator can find chemical concentration data for an ecosystem comparable to their site, then bootstrap resampling could be conducted with that data, and sample sizes selected accordingly. However, there are other approaches that should also be considered when site-specific data are limited.

3.2.1.2 Monte Carlo BAF Analysis

Monte Carlo methods are used to *simulate* data using assumptions about the probability distribution(s) of random variables (Metropolis and Ulam, 1949; Robert and Casella, 2004). The investigator can use Monte Carlo to simulate chemical concentrations for biota and water when sufficient data are not available for the site of interest. To do so, the investigator should fit an appropriate distribution (i.e., normal, lognormal, uniform) to the available data for the chemical concentrations in biota and water. Simulated (or synthetic) data can then be generated and substituted for the chemical concentration measurements. The same Bootstrap resampling procedure (Section 3.3.1) can then be applied to estimate how the precision of the BAF depends upon the number of chemical concentration measurements. Monte Carlo analysis is demonstrated by examples in Appendix 3C, based on the same data used for the Bootstrap resampling method (Green Bay Mass Balance data for PCB congeners 18, 52, 149 and 180 in zone 3) so that the results of the two methods could be directly compared.

In general, the Monte Carlo analysis of BAF precision as a function of sample sizes produced results comparable to those obtained via Bootstrap resampling. For moderately variable chemical concentrations, the ratio of the 90th to the 10th percentile values of the distribution of BAFs (confidence limit ratios, CLRs) were mostly 5 or less. BAF confidence limit ratios decline predictably as the number of water and/or fish samples increase, although once the number of samples exceeds about 6, the reductions in BAF confidence limit ratios become incrementally much smaller. Depending upon the requirements for BAF accuracy, exceeding sample sizes of 10 appears to be warranted only for sites having very high variability in chemical concentrations in fish and/or water. It is most effective for the investigator to apply more sampling effort to the chemical concentration (biota vs. water) that is more variable. Correlations between chemical

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concentrations in biota and water were found to be beneficial in terms of reducing the BAF confidence limit ratios, percent bias and root mean square errors (RMSE). The benefits increased with the magnitude of the correlation, and were more beneficial in terms of reducing BAF uncertainty for smaller sample sizes. Mis-specifying the concentration distribution (i.e., specifying a lognormal concentration distribution when the actual distribution was normal) had little or no effect on the outcome of the Monte Carlo analysis. However, the investigator should always use averaging methods appropriate to the concentration data, for example applying the lognormal transformation only when justified.

Both Bootstrap resampling and Monte Carlo analysis demonstrate that compositing of water and/or fish samples for analysis is highly effective for reducing the cost of chemical analyses, assuming that composite samples are formed appropriately (see Sections 3.3.5 and 3.4.5). A single measured concentration from a well-formed composite is equivalent to the arithmetic average of concentrations measured in samples used to form the composite. In fact, the numerical analyses demonstrate that, from the standpoint of measuring a BAF, sample compositing does not reduce the accuracy of the measurement at all.

3.2.2 Modeling Simulation of BAF Sampling Designs

Once the appropriate number of biota and water samples to collect has been determined, the investigator should allocate the samples both spatially and temporally, in order to complete the sampling design. Burkhard (2003) performed model simulations to explore how the variabilities in water and sediment chemical concentrations translate into the variabilities associated with BAFs (and BSAFs, which will be discussed separately in Section 4) based upon different sampling designs. Rather generic conditions and simple models were used, so that the connections between input and resulting variabilities in BAF designs were straightforward and apparent. The approach used for the model (i.e., a river segment with a food web consisting of four trophic levels) is a variant of that developed by Thomann et al. (1997). Details of the modeling approach, and presentation and discussion of the results, are presented in Appendix 3D.

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3.2.2.1 Using Model Simulations to Develop Field-Sampling Designs

The kinetics of chemical uptake and loss by the fish (or other aquatic organism) controls the chemical residue that resides in the organism. These kinetic processes are directly dependent upon the chemical's hydrophobicity and metabolism rate in the fish. Successful field-sampling designs should account for the chemical uptake and loss kinetics, and for the changes in chemical concentrations occurring in the fish's environment. The modeling simulations strongly demonstrate that smaller uncertainties can be obtained by using properly developed sampling design structures. The haphazard collection of samples for the measurement of BAFs can, and most often will, result in highly uncertain BAF values. Consequently, such measured values will have poor predictive power.

Burkhard's (2003) simulations provide substantial insight into what an appropriate sampling design structure might be for BAF measurements:

- For chemicals with log K_{ow} of 4 and less with any rate of metabolism, the concurrent collection of fish and water samples over time provides the smallest degree of uncertainty for BAF measurements; the spacing of sampling events does not seem critical.

- For nonmetabolizable and slowly metabolizable chemicals with log K_{ow} of 5 and greater, practically identical BAF uncertainties are obtained using sampling designs consisting of the collection of a series of water samples over time, and either the concurrent collection of fish samples with each water sample or the collection of fish samples with the last water sample. From a field-sampling perspective, the one-time collection of fish is appealing because of the logistics of assembling field-sampling crews.

- In contrast to chemicals with lower K_{ow}, nonmetabolizable chemicals with log K_{ow} of 6 and greater require numerous water samples spaced widely apart over time to obtain lower uncertainty in the BAF measurement. The spacing and timing of fish collection is again relatively unimportant.

- With increasing chemical metabolism rate, appropriate sampling design structures transition from the numerous water samples spaced widely over time (with concurrent fish collection with the last or all water samples) to the designs appropriate for lower K_{ow} chemicals; that is, concurrent collection of water and fish samples over time with sample spacing not being very critical.

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This transition in appropriate sampling design structures suggests that the concurrent sampling design is a more robust or universally applicable design because it can be used for chemicals of all hydrophobicities and all metabolism rates. This advantage for the concurrent sampling design will be especially useful when information is lacking on the chemical's metabolism rate in the fish, a situation that exists for nearly all nonionic organic chemicals.

For sampling to determine BAFs, chemicals with large K_{ow}s will generally require that numerous water samples be averaged over time to establish the long-term chemical concentrations in the water. In contrast, for chemicals with low K_{ow}s, because the concentrations in the fish mimic those in water, the time scale for establishing the chemical concentrations in the water shrinks to concurrent sampling of both fish and water. For less hydrophobic chemicals, current chemical concentrations in the water provide a good predictor of the chemical concentration in the fish.

Chemicals with intermediate metabolism rates and mid-range hydrophobicities (log K_{ow}s of 4 to 6) present one of the more difficult challenges in selecting an appropriate sampling design structure for measuring BAFs. This range of hydrophobicities lies within the transition zone between the much more obvious design structures appropriate for low and high K_{ow} chemicals.

The process for developing successful field-sampling structures for BAF measurements can primarily focus upon three parameters: temporal variability, metabolism, and K_{ow}. These three parameters can range widely, and depending upon their values, require dramatically different field designs. The greatest number of samples would be required for high-K_{ow} chemicals in aquatic ecosystems subjected to extremely high temporal variability in chemical concentrations in water. Although spatial variability has been discounted as a dominant factor in sampling design, knowing or understanding the immediate home range of the sampled organisms is required. Without this information, one cannot ascertain whether the collected water samples are reflective of the actual water exposure history for the sampled organisms. Poor spatial coordination of fish samples with their actual water exposures will yield BAFs with poor accuracy and large biases.

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The sampling structure (i.e., number of sampling events over time and space) can be developed for the chemical and ecosystem of interest based upon the modeling simulations. By using the modeling results from Appendix 3D as a guide, some illustrative BAF sampling structures have been developed (Table 3-1). These illustrative designs provide a sense of how sampling design structures might be influenced by differences in temporal variabilities, metabolism rates, and K_{ow}s. Alternative sampling designs can provide similar uncertainties. The illustrative designs incorporate some considerations from a field implementation perspective (for example, collection of water samples once a week as opposed to every 3 or 5 days) and are based upon a continuum or gradient of modeling responses. As indicated above, the total number of samples required for a successful measurement is dependent upon the desired precision, and, thus, the illustrative sampling structures suggest the number and spacing of sampling events for a field study, and not the total number of samples needed for the study.

The effects and importance of the immediate home range of the fish are not included in the illustrative sampling structures in Table 3-1. Although spatial variability of the chemical in the ecosystem is not directly included in the illustrative sampling structures, sample collection for each sampling event should span the home range of the organisms in the ecosystem. Home range depends upon the species , although larger fishes tend to have larger home ranges (see Section 3.3.2). Information about the home range of the fish leads to an assessment of where the fish resides relative to the spatial variability in chemical concentrations. By collecting samples across the organism's home range, a truer picture of the average chemical exposures to the organisms of interest will be obtained. The ideal situation for measuring a BAF is when there are minimal concentration gradients across the organism's home range. However, spatial variability in the concentrations of the chemical does not add large uncertainties into the measured BAF beyond those caused by temporal variability of the chemical concentrations in the water. Further, bioaccumulation simulations for migrating fish suggest that BAFs can be measured with low uncertainty even when extreme spatial concentrations exist at the field site, provided the measurements are performed in more contaminated locations of the site for more hydrophobic chemicals, i.e., log K_{ow}>5 (Burkhard, 2003).

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In addition to the home range issue above, the illustrative BAF sampling structures (Table 3-1) do not include the effects of collecting composite water samples over time. For higher K_{ow} chemicals, compositing reduces the uncertainty in the BAF measurement, whereas the uncertainty in the BAF measurement is increased by compositing for lower K_{OW} chemicals.

3.2.3 How Can These Methods be used to Help Design a BAF Sampling Plan?

As indicated in Section 3.2.1, the total number of samples required for a successful BAF measurement is dependent upon the precision desired by the investigator. On the other hand, the illustrative sampling structures in Table 3-1 suggest the number and spacing of sampling events for a field study, but not the necessary number of samples. The results of bootstrap resampling or Monte Carlo analysis can be used together with modeling simulations as the basis for a rational sampling design process. The design process is outlined below.

Table 3-1. Illustrative Bioaccumulation Factor Sampling Design Structures. Uncertainties Associated With Design Structures Are Ecosystem Specific

log K_{ow}	Metabolism Rate	Sampling Design[a]	Sampling Events	
			Minimum number (depends on temporal variability)	Minimum spacing between sampling events (d)
≤ 3	low	2nd series	1	7
4	low	2nd series	2	7
5	low	1st or 2nd series	1, 3, 6[b]	7
≥ 6	low	1st or 2nd series	1, 5, 8[b]	30
≤ 4	medium	2nd series	1	7
5	medium	1st or 2nd series	1, 2, 3[b]	7
≥ 6	medium	1st or 2nd series	1, 4, 6[b]	30
all	high	2nd series	1	7

[a] 1st series = collection of a series of water samples with the collection of fish samples concurrently with the last water sample; 2nd series = collection of paired fish and water samples with each sampling event.
[b] Values are ordered according to low, medium, and high temporal variability, respectively.

1. The investigator determines the goal for accuracy of the BAF measurement, and expresses this goal as the ratio of 90% confidence limits. In the examples presented in Appendices A and C, the BAF confidence limit ratios typically ranged from about 2 to 12.

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2. The investigator determines the number of biota and water concentration samples to collect. Suggestions are offered in Appendices A and C for determining the numbers of samples to collect.

   a) If site-specific data or data representative of the site and chemical are available, bootstrap resampling can help determine sample numbers;

   b) If more representative data are not available, Monte Carlo analysis as demonstrated by example in Appendix C may be useful.

   c) The investigator should avoid collecting fewer than 6 concentration samples in biota and (especially) water, unless significant uncertainty in the BAF is acceptable.

3. The investigator selects an appropriate sampling design structure. Suggestions are offered in Section 3.2.2.1 based on:

   a) Chemical factors: hydrophobicity (log K_{ow}) and rate of metabolism; and

   b) Temporal variability of water concentrations, based upon factors of the waterbody at the site.

   Table 3-2 illustrates the relationship between categories of waterbodies (lakes and reservoirs, estuaries and tidal rivers, rivers and streams) and the degree of temporal variability in concentrations observed for various chemicals. The coefficient of variation (CV) for the chemical concentrations generally increase as one moves from quiescent waterbodies towards those that are more advective (flowing) with shorter hydraulic residence times. Therefore, the investigator can use the waterbody categories in Table 3-2 to estimate the temporal variability of water concentrations.

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4. The investigator allocates the number of samples (based upon guidance from Step 2) evenly among sampling events (determined in Step 3).

Table 3-2. Waterbody Type as Indicator of Temporal Concentration Variability[a]

low variability < - > high variability					
Lakes and Reservoirs		**Estuaries and Tidal Rivers**		**Rivers and Streams**	
Waterbody	**CV[b]**	**Waterbody**	**CV**	**Waterbody**	**CV**
Lake Michigan (dissolved PCBs)	**0.37**	**Hudson River (Aroclor 1254)**	**0.60**	**Mississippi River, MS (chloroform)**	**0.97**
		James River estuary (Kepone)	**0.60**	**Naugatuck River, CT (dissolved copper)**	**0.60**
		Green Bay zone 3 (PCBs)	**0.6 - 0.8**	**lower Fox River, WI (dissolved PCBs) congeners 28+31** **congener 149** **congener 180**	**0.54 – 0.57** **0.37 – 1.11** **0.55 - 2.19**
				Lake Michigan Tributaries (PCBs)	**0.19 – 1.50**

[a] Variability of chemical concentrations may be higher at concentrations approaching the limit of detection.
[b] CV = coefficient of variation.

3.3 MEASURING CHEMICAL CONCENTRATIONS IN BIOTA

This section describes the development of a field plan for sampling biota to support the measurement of a site-specific BAF. This is based upon a number of documents, including USEPA (2000b), USEPA (1997a), Versar (1982) and USEPA (1997b; Section 4.2). These documents provide more detailed guidance on the sampling design of field studies and recommend field procedures for collecting, preserving, and shipping samples to a processing laboratory for target analyte analysis. Planning and documentation of all field procedures ensures that collection activities are cost-effective and that sample integrity is preserved during all field activities. EPA's systematic planning tool is the Data Quality Objectives (DQO) process. The

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elements of systematic planning are stated in Chapter 3 of the *EPA Manual 5360 - EPA Quality Manual for Environmental Programs* (USEPA, 2000d) and include:

- Identification and involvement of the project manager, sponsoring organization and responsible official, project personnel, stakeholders, and experts, etc. (e.g., all customers and suppliers);
- Description of the project goals, objectives, and questions and issues to be addressed;
- Identification of project schedule, resources (including budget), milestones, and any applicable requirements (e.g. regulatory requirements, contractual requirements);
- Identification of the type of data needed and how the data will be used to support the project's objectives;
- Determination of the quantity of data needed and specification of performance criteria for measuring quality;
- The data will be obtained (including existing data) and identification of any constraints on data collection;
- Specification of QA and QC activities to assess the quality performance criteria;
- Description of how the acquired data will be analyzed, evaluated and assessed against its intended use and the quality performance criteria.

The investigator and field sampling staff should develop a detailed sampling plan prior to initiating a field study. As described by EPA (USEPA, 2000b), there are seven major parameters to be specified prior to the initiation of any field biota sample collection activities:

- Target analytes and analytical methods
- Target species (and size classes)
- Sampling locations
- Sampling times
- Sample type (whole organism or edible portion)
- Replicate samples
- Sample collection methods

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The role of each of these parameters in developing an appropriate field plan for biota sampling is discussed below.

3.3.1 Target Analytes and Analytical Methods

Site-specific BAFs are measured in order to support the derivation of AWQC for specific contaminants. Knowing the chemical of concern, the investigator should make a number of decisions regarding the analysis of tissue samples. This includes considering whether analytical alternatives exist for measuring the chemical of concern, and if so, which method is most appropriate. In addition, it may be necessary to specify which form of the chemical should be measured. Furthermore, it is important to analyze tissue samples using methods that are chemical-specific, sensitive, accurate and precise.

Bioaccumulation factors should only be determined for individual chemicals. In cases where the chemical of interest is a mixture (e.g., PCBs, chlordane), the study design will require that individual chemicals composing the mixture of interest be quantified individually. This will result in BAFs for individual components of the chemical mixture. This requirement is necessary because individual chemicals in a mixture usually behave differently in the environment (i.e., different portions of the individual components of the mixture will be present in different amounts among the sediment, water and biota). The investigator must select analytical methods that are specific for the individual chemical (or chemicals, in the case of a mixture) of interest.

In addition, the analytical method should be sensitive enough to quantify the chemical concentrations in both biota and water. Examples of highly-bioaccumulative chemicals that are difficult to quantify in water include a number of toxicologically-significant polychlorinated dibenzodioxin (PCDD) congeners. Unless the investigator is confident that chemical concentrations can be quantified in the majority (preferably > 80%) of both biota and water samples, a different approach of determining BAFs should be considered.

It is also important to measure other parameters that are significant to the bioaccumulation process. For nonionic organic chemicals, lipid content of the target species should be measured in the same tissue in which the contaminant was measured to permit lipid

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normalization. This will usually be the fillet for finfish and the edible tissue for shellfish (see subsection 3.3.2).

3.3.2 Target Species and Size Class Selection

The investigator should specify the aquatic organisms to be targeted for sampling, as well as the size ranges to collect. The target species and sizes selected for sampling should be commonly consumed locally and of harvestable size. State fishing regulations may be a source of useful information and may be especially important to consider when compositing fish. Other aspects of the field sampling plan will follow from identifying the target species. For example, the home range of the target species will dictate the spatial scale of the sampling effort.

Several biological attributes of the target species should also be considered when sampling target species for BAF determinations used in deriving human health criteria. For example, the size/age of the organism can affect the extent of bioaccumulation in the organism. Young fish can exhibit lower accumulation of some contaminants due to growth dilution. In addition, the reproductive status (e.g., pre/post spawning) can alter the body burden of contaminants, with significant contaminant loss observed due to maturation and release of sperm or eggs. The investigator should consider determining the age of sampled fish because older fish tend to accumulate higher concentrations for many chemicals, and changes in behavior (movement, migration, diet) are often related to organism age. The size of the target species should be representative of the size being consumed by the target human population. If this size range is broad, stratifying sampling strategies by size class is necessary, particularly when taking composite samples. The timing of sampling should include the period of most frequent harvesting of the species.

In freshwater ecosystems, one bottom-feeding and one predator fish species should be collected. In estuarine/marine ecosystems, either one bivalve species and one finfish species or two finfish species should be collected. Second- and third-choice target species should be selected in the event that the recommended target species cannot be successfully collected at the site. The same criteria used to select the recommended target species should be used to select alternate target species.

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The correlation between increasing size (age) and contaminant tissue concentration observed for some freshwater finfish species (Voiland et al. 1991) may be much less evident in estuarine/marine finfish species (Pollock, 1993). The movement of estuarine and marine species from one niche to another as they mature may change their exposure at a contaminated site. The size of estuarine/marine target species collected should still be representative of the size being consumed by the target human population.

The trophic level of the fish species sampled should be determined by taking into account the life stage(s) consumed and food web structure at the location(s) of interest. Site-specific data, such as gut contents or stable isotope analyses, are preferred for determining trophic levels. Jardine et al. (2006) provide guidance on the application and interpretation of stable isotope ratios to measure trophic levels. The investigator should also consult with fisheries experts and refer to life history literature for the species of interest when making trophic level determinations. Most often the field studies used will be those that include fish at or near the top of the aquatic food chain (i.e., in trophic levels 3 and/or 4). In situations where consumption of lower trophic level organisms represents an important human exposure route, such as certain types of shellfish at trophic level 2, the field study should also include appropriate target species at this trophic level.

Behavior and life cycle aspects are important because they can significantly affect exposure and overall bioaccumulation. For each target species, the investigator should address the following questions.

- *Do the species of interest migrate?* If the answer is yes, the investigator should know the approximate arrival and departure dates for the organisms of interest for your site.

- *Are there multiple populations of the organisms of interest?* If the answer is yes, which population does the investigator wish to sample? Populations may exhibit different behavior characteristics (e.g., migration, range, habitat, and feeding preference), which can lead to differences in chemical exposure and bioaccumulation.

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For example, two populations of a fish species may both inhabit a tributary - one that migrates seasonally between the tributary and the adjacent embayment, and a resident population that does not migrate. If the tributary is highly contaminated by a bioaccumulative chemical relative to the embayment, chemical concentrations will probably be higher in the resident fish. In this example, the investigator should target the collection of fish from the resident population in order to measure the site-specific BAF for the tributary. This could be accomplished by sampling when the migratory population is absent. The field plan should take the behavioral differences of multiple populations into account, in order to sample organisms from a specific population. Otherwise, it may be difficult or impossible to distinguish which population(s) are represented by the sampled organisms.

- *What is the home range size for the target species at your site?* Depending upon the site, the degree of difficulty in defining the immediate home range of the organism can vary widely. In situations where the movement of the organisms is confined by the geography of the site (e.g., dams or falls) the home range of the organisms can probably be defined fairly easily. In estuaries, the home range of some fish species are constrained by salinity gradients. Home ranges can be determined by tagging/recapture, radio-telemetry, and/or ultrasonic telemetry studies at the site of interest. Valuable information can be obtained from fisheries biologists or recreational fishermen that are familiar with the waterbody and fish species. Home ranges for freshwater fishes can also be estimated using the allometric relationships of Minns (1995):

$$H = \exp\left[-2.91 + 3.14 \cdot HAB + 1.65 \cdot \ln(L)\right] \qquad \text{(Equation 3-5a)}$$

or

$$H = \exp\left[3.33 + 2.98 \cdot HAB + 0.58 \cdot \ln(W)\right] \qquad \text{(Equation 3-5b)}$$

where:

H	=	home range size (m^2)
HAB	=	0 for rivers and 1 for lakes
L	=	body length (mm), and
W	=	organism body weight (g).

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An example of applying equation 3-5 is presented below. For freshwater invertebrates, and for marine and estuarine ecosystems, allometric relationships for home range have not been reported.

Estimating the home range for freshwater fish

The bluegill sunfish is a common inhabitant of small lakes and creeks. A representative length for an adult bluegill is 180 mm (7 inches). Using equation 3-5a, we can estimate the home range for a bluegill sunfish in a lake:

$$H = \exp[-2.91 + 3.14 \cdot HAB + 1.65 \cdot \ln(L)]$$

$$= \exp[-2.91 + 3.14 \cdot 1.0 + 1.65 \cdot \ln(180\ mm)]$$

$$= \exp(8.80) = 6{,}624\ m^2$$

According to equation 3-5a, the estimated home range of a bluegill sunfish in a lake is 6,624 m^2 (1.6 acres).

To estimate the home range in a river, a HAB of 0 would be substituted for the value of 1.0 used above in equation 3-5a:

$$H = \exp[-2.91 + 3.14 \cdot 0 + 1.65 \cdot \ln(180\ mm)] = \exp(5.66) = 287\ m^2$$

According to Carlander (1969), the home range for this species is considerably smaller than the estimates calculated above: typically less than 0.25 acres (~1,000 m^2) in lakes and not exceeding 30 meters in streams.

Having a good understanding of the immediate home range of the species is important. Organisms with smaller home ranges will be more representative of the study site than those with large home ranges which extend beyond the study site. Just because an organism is caught at a sampling location, one can not infer that the chemical residue in the fish are due to the chemicals residing at the study site. Knowledge of the organism's home range is the only way that the investigator can

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establish the connection of the fish (or other aquatic organism) to the sampling location.

It is very useful to consult with local fisheries experts during the sampling design phase of the field study to help in determining the immediate home range, diet and trophic level of the organisms at the site. Although the above allometric relationships are available for estimating home ranges, the investigator should not necessarily assume that the "calculated" and "actual" immediate home ranges for the organisms are the same. The investigator will still need to determine, to the extent possible, the immediate home ranges for the organisms at the site.

- *Do the organisms of interest exhibit diel (day/night) behavior in habitat at your site?* If the answer is yes, the sampling plan should reflect the typical behavior for the species of interest.

Behavioral information for the species of interest will be used to specify the location and timing of field sampling for biota, as discussed in the following 2 subsections.

3.3.3 Sampling Locations

Selection of biota sampling locations may be quite straightforward where the source of pollutant introduction is highly localized, or if site-specific hydrologic features create a sink where chemically contaminated sediments accumulate and the bioaccumulation potential might be enhanced. Upstream and downstream water quality and sediment monitoring stations/locations bracketing point source discharges, outfalls, and regulated disposal sites can often be used to characterize the geographic extent of the contaminated area. Within coves or small embayments where streams enter large lakes or estuaries, the geographic extent of contamination may also be characterized via multilocational sampling to bracket the areas of concern. Such sampling designs are clearly most effective where the target species are sedentary or of limited mobility (Gilbert, 1987). In addition, the existence of barriers to migration, such as dams, should be taken into consideration. Selection of sampling sites should also consider temporal and spatial variations in food web structure that may occur across the study area.

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Site selection considerations should also include the number of samples necessary to characterize different waterbody types (lakes, rivers, estuaries, and coastal marine waters) based on hydrodynamics (including waterbody size). Typically, as the size of a waterbody increases (from small lakes to larger lakes to Great Lakes; or from streams, to rivers, to estuaries, to coastal marine waters), more samples are needed to achieve a specific precision. For example, fish inhabiting relatively small lakes are likely to be exposed to a relatively homogeneous aquatic environment of contaminant concentrations. In a riverine, estuarine, or coastal situations, however, the hydrodynamics of the ecosystem can greatly affect the magnitude and nature of chemical exposure in the water. Thus, the amount of time that any fish spends exposed to the contamination may be highly variable as compared to the relatively homogeneous exposures that might occur in smaller, less hydrologically dynamic lake ecosystems. Large sites with strong spatial gradients may require spatially-stratified sampling designs. Guidance on optimizing data collection options, via different probability or authoritative sampling designs, is offered in USEPA (2002a).

The investigator should also consider the inherent migratory nature of the species when determining the number of samples to collect. Smallmouth bass, a riverine species, have a home range of 500 to 4,500 m^2, but typically migrate up to 45 km (28 miles) (Reid and Rabeni, 1989). In contrast, many Great Lakes fish species, as well as riverine, estuarine, and marine species, migrate considerable distances during spawning periods. Several Great Lakes species also move upstream considerable distances into tributary rivers to spawn. Lake trout in the Great Lakes have been found to migrate up to 300 km (200 miles) with larger fish migrating 300 miles (480 km) (Daly et al. 1962). For many marine species, estuaries are the spawning areas for the adults and nursery areas for the developing juveniles, who eventually travel offshore as adults and return again to the estuaries to spawn. For these species, migratory or seasonal movements can take place both from inshore to offshore areas and along the coasts. Obviously, the number of samples needed to calculate an accurate average chemical concentration for bluegill sunfish inhabiting a relatively homogeneous environment with respect to contaminant concentrations is quite different from that required for the more mobile species like the smallmouth bass and lake trout.

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Home range should also be considered for shellfish. Bivalve molluscs, like the oyster or mussel, cement themselves to hard substrates as young spat and are then unable to move. Although clams and scallop species are slightly more mobile, they also typically stay in the general area in which they first settled out of the water column. For crustaceans like the blue crab and lobsters, however, movements both into and out of estuaries as well as into deeper water offshore are possible. As the complexity of the hydrodynamics of an ecosystem increases and the mobility of the target species increases, so too do the number of samples and the number of sampling stations required to accurately determine the average chemical concentration at the site.

Biota sampling should be conducted in frequently fished areas, possibly including the following locations.

Areas near point source discharges such as

- industrial or municipal discharges,
- combined sewer overflows (CSOs),
- urban storm drains, and
- urbanized embayments or tributaries in large estuary and lake systems.

Areas near nonpoint source inputs such as

- landfills, Resource Conservation and Recovery Act (RCRA) sites, or Superfund Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) sites,
- areas of intensive agricultural, silvicultural, or resource extraction activities,
- areas of urban/suburban land development,
- areas subject to significant shoreline or bank erosion and/or interactions between a river and adjacent floodplains, and
- areas receiving inputs through multimedia mechanisms such as hydrogeologic connections or atmospheric deposition.

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Areas acting as potential pollutant sinks where contaminated sediments accumulate and bioaccumulation potential might be enhanced, such as

- areas where water velocity slows and organic-rich sediments are deposited, such as near the inside bank of stream or river bends, deep pools, and impoundments above dams,
- areas of ponds and lakes where water depths exceed the wave "base" that limits sediment resuspension due to the oscillatory shear stresses from wind waves, and
- the convergence zone between fresh and saltwater in estuaries.

Identifying sampling locations near significant point source discharges are usually straightforward. It is often more difficult, however, for the investigator to identify clearly defined sampling locations in areas affected by pollutants from nonpoint sources. For these sites, assessment information summarized in state Section 305(b) reports should be reviewed before locations are selected. State 305(b) reports are submitted to the EPA Assessment and Watershed Protection Division biennially and provide an inventory of the water quality in each state. The 305(b) reports often contain Section 319 nonpoint source assessment information that may be useful in identifying major sources of nonpoint source pollution to state waters. States may also use a method for targeting pesticide hotspots in estuarine watersheds that employs pesticide use estimates from NOAA's National Coastal Pollutant Discharge Inventory (Farrow et al. 1989).

In addition to the intensity of subsistence, sport, or commercial fishing, factors for evaluation when selecting fish and shellfish sampling sites include (Versar, 1982):

- proximity to water and sediment sampling sites
- availability of data on fish or shellfish community structure
- bottom condition
- type of sampling equipment
- accessibility of the site

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The most important benefit of locating fish or shellfish sampling sites near sites selected for water and sediment sampling is the possibility of correlating contaminant concentrations in different environmental compartments (water, sediment, and fish). Selecting sampling sites in proximity to one another is also more cost-effective because it provides opportunities to combine sampling trips for different matrices.

The availability of data on the indigenous fish and shellfish communities is a consideration when selecting sites. Information on preferred feeding areas and migration patterns is valuable in locating populations of the target species (Versar, 1982). Knowledge of habitat preference provided by fisheries biologists or commercial fishermen may significantly reduce the time required to locate a suitable population of the target species at a given site.

One additional consideration associated with sample site selection is whether the sampling area includes waters inhabited by threatened or endangered species. If such waterbodies are to be monitored, the investigator should obtain a permit from the U.S. Fish and Wildlife Service (USFWS) if their sampling effort could potentially impact a freshwater species or from the National Marine Fisheries Service (NMFS) if their sampling effort could potentially impact any marine or anadromous species covered under the Endangered Species Act (ESA) of 1973. A species is listed under one of two categories, endangered or threatened, depending on its status and the degree of threat it faces. An endangered species is one that is in danger of extinction throughout all or a significant portion of its range. A threatened species is one that is likely to become endangered in the foreseeable future. The USFWS maintains a list of all plant and animal species native to the United States that are candidates or proposed for possible addition to the Federal List. A complete listing of the current status of all threatened and endangered species as well as information about each USFWS region is available on-line on the USFWS website at http://endangered.fws.gov/wildlife.html. Additional information can be gleaned by contacting the specific USFWS regional office that has primary responsibility for an endangered species.

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3.3.4 Sampling Times

Selection of the most appropriate times for sampling is very important, particularly when target species will be sampled on only a few occasions. Sampling should be conducted during the period when the target species is most frequently harvested (USEPA, 1989a; Versar, 1982) but should also consider the reproductive cycle and lipid dynamics of the target species, which may be related to temperature as well as season. In fresh waters the most desirable sampling period may be from late summer to early fall (i.e., August to October) (Phillips, 1980; Versar, 1982). The lipid content of many species is generally highest at this time. Also, water levels are typically lower during this time, thus simplifying collection procedures. The late summer-early fall sampling period may not be appropriate, however, if it does not coincide with the legal harvest season of the target species, and/or the target species spawns during this period[3]. A third exception to late summer-early fall sampling concerns monitoring for the organophosphate pesticides. Sampling for these compounds is best if conducted during late spring or early summer within 1 to 2 months following pesticide application because these compounds are degraded and metabolized relatively rapidly compared to organochlorine pesticides. However, the target species should be sampled during the spring only if the species can be legally harvested at this time. It should also be noted that sampling considerations for aquatic and aquatic-dependent wildlife could be different from that for the protection of human health.

In estuarine and coastal waters, the most appropriate sampling time is during the period when most fish are caught and consumed (usually summer for recreational and subsistence fishers). For estuarine/marine shellfish (bivalve molluscs and crustaceans), two situations may exist. The legal harvesting season may be strictly controlled for fisheries resource management purposes, or harvesting may be open year round. In the first situation, shellfish contaminant monitoring should be conducted during the legal harvest period. In the second situation, monitoring should correspond with the period when the majority of harvesting is conducted during the legal season. The investigator should consider different sampling times for target shellfish species if differences exist between the commercial and recreational harvesting periods.

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[3] If the target species can be legally harvested during its spawning period, then sampling to determine contaminant concentrations can be conducted during this time.

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Ideally, the investigator selects a sampling period that avoids the spawning period of the target species, including the period one month before and after spawning, because many aquatic species are subject to stress during spawning. Tissue samples collected during this period may not be representative of the normal population. For example, feeding habits, body fat (lipid) content, and respiration rates may change during spawning and may influence pollutant uptake and clearance. The collection of samples may also adversely affect some species, such as trout or bass, by damaging the spawning grounds. Most fishing regulations protect spawning periods to enhance propagation of important fishery species. Species-specific information on spawning periods and other life history factors is available in numerous sources (e.g., Carlander, 1969; Emmett et al. 1991; Pflieger, 1975; Phillips, 1980). In addition, digitized life history information is available in many states through the Multistate Fish and Wildlife Information Systems (1990) on the web at http://fwie.fw.vt.edu.

Sample timing for species that migrate into and out of the site is a particular concern. EPA's preferred method of accounting for organism migration is to collect aquatic organisms just before they migrate back out of the site. This approach maximizes the amount of time the organism spends at the site of interest, and provides the best estimate of the residue in the organism based upon the organism's exposure at the site. If the organisms spend a very short time at the site (e.g., the fish migrate through the site in a few days to a week), the determination of a BAF for that species is not useful, even if the BAF can be measured. A site-specific BAF measured in this way may be biased because the chemical concentrations measured in water from the site would not be reflective of the organism's recent exposure history. The degree of bias will be related to the uptake and elimination kinetics (related to hydrophobicity) and the metabolism rate of the chemical, as discussed in Section 3.2.2.

3.3.5 Sample Type

EPA has recommended composite samples of fish fillets or of the edible portions of shellfish for analysis of chemicals of concern in bioaccumulation studies (USEPA 1987; 1989a). Composite samples are homogeneous mixtures of samples from two or more individual

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organisms of the same species collected at a particular site and analyzed as a single sample. Because the costs of preparing and analyzing individual samples are higher than the costs of preparing and analyzing a composite sample, the latter sample type is the most cost-effective for estimating average tissue concentrations in target species populations. For a well-formed composite, a single measured concentration should be similar to the arithmetic average of concentrations for the individual organisms within the composite. Compositing may be necessary in order to collect sufficient tissue for chemical analysis of smaller organisms. Even for larger organisms such as predator fish, however, compositing offers advantages over sampling and analyzing individual organisms. Most importantly, composites formed from individual organisms collected at a specific time and place will avoid sampling the variability in chemical concentrations between individuals, which can be a significant component of the overall variability. Since BAFs measured for use in deriving national recommended ambient water quality criteria for protecting human health are intended to represent average, long-term chemical concentrations, it is neither necessary nor desirable to sample variability in chemical concentrations among individual organisms. Although composite sampling will not reveal extreme contaminant concentration values in individual organisms, this is not considered a major disadvantage given the goal of measuring average chemical concentrations for BAF determination.

Fish samples should reflect how the target population commonly prepares fish for consumption. The investigator should select a composite sample type for chemical analysis based on the tissue types and preparation methods of fish consumed by the target population of concern. For example, few consumers in the general population eat the skin of the fish, which may justify its removal for analysis. Analysis of skinless fillets may also be more appropriate for some target species such as catfish and other scaleless finfish species. In contrast, using whole fish with skin-on as the sample type for assessing PCBs, dioxins/furans, or organochlorine pesticide exposures in populations of Native Americans, Asian Americans, Caribbean-Americans, or other ethnic groups that consume whole fish in a stew or soup is warranted because these contaminants accumulate in fatty tissues of the fish. Cooking the whole fish to make a stew or soup releases the PCBs, dioxins/furans, or organochlorine contaminants into the broth; thus, analysis of whole fish should mirror the way the consumer prepares the fish.

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Similarly, the investigator should consider whether using skin-on fillets (with belly-flap included) is appropriate for the general fishing population, since this is a standard filleting method among recreational fishers. This method also allows for the inclusion of the fatty belly flap tissue and skin in which hydrophobic, nonionic organic chemicals such as PCBs and dioxins/furans concentrate. It also takes into account the fact that some consumers may not neatly trim the more highly contaminated fatty tissue from the edible muscle fillet tissue. Additional guidance regarding site-specific fish consumption is provided in Section 4.3.3.1 of the 2000 Human Health Methodology.

In any study design, it is important that biota samples be collected and composited in size/age classes. For fish, dietary composition changes substantially with size/age, and these changes can result in differences in BAFs among age classes. For forage fish, common classes are young-of-the-year (YOY), juveniles, and adults. For piscivorous fishes, common classes are year classes (e.g., 2, 3, 6, and 10 years old). The investigator should consider the following guidelines for the compositing of biota samples.

- *Composited organisms must all be of the same species:* Individual organisms combined to form composite samples must be of the same species because bioaccumulation potential can vary among different species. Accurate taxonomic identification prevents the mixing of closely related species with the target species. Individuals from different species should not be combined to form a composite sample (USEPA, 1989a, 1990).

- *The composited organisms should all satisfy any legal requirements of harvestable size or weight, including "slot-limit" restrictions*[4]*:* Alternatively, they should at least be of consumable size if no legal harvest requirements are in effect.

- *Composite samples should be comprised of equal weights of ground tissue from each organism:* Samples comprised of equal tissue weights from each organism will provide

[4] A slot limit is a protected size range (e.g., lengths between 15" and 24") requiring the release of fish within the specific range. Fish smaller or larger than the "slot" may be harvested.

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the least-biased average of concentrations for the individual organisms within the composite.

- *Composited organisms should be of similar size:* Individual organisms used in composite samples should be of similar size (WDNR, 1988). For fish or shellfish, the total length (or size) of the smallest individual in any composite sample should be no less than 75 percent of the total length (or size) of the largest individual in the composite sample (USEPA, 1990). For example, if the largest fish is 200 mm, then the smallest individual included in the composite sample should be at least 150 mm. In the California Mussel Watch Program, a predetermined size range (55 to 65 mm) for the target bivalves (*Mytilus californianus* and *M. edulis*) is used as a sample selection criterion at all sampling sites to reduce size-related variability (Phillips, 1988). Similarly, the Texas Water Commission (1990) specifies the target size range for each of the recommended target fish species collected in the state's fish contaminant monitoring program.

  For persistent chlorinated organic compounds (e.g., DDT, dioxin, PCBs, and toxaphene) the larger (older) individuals within a population are generally the most contaminated (Phillips, 1980; Voiland et al. 1991). As noted earlier, this correlation between increasing size and increasing contaminant concentration is most striking in freshwater finfish species and is less evident in estuarine and marine species. Size is used as a surrogate for age, which provides some estimate of the total time the individual organism has been at risk of exposure. Therefore, the primary target size range ideally should include the larger individuals harvested at each sampling site.

- *Composited organisms should be collected at the same time:* Individual organisms used in a composite sample ideally should be collected at the same time (i.e., collected as close to the same time as possible but no more than 1 week apart). This is done to minimize temporal changes in contaminant concentrations (e.g., associated with the reproduction cycle of the target species). If organisms used in the same composite are collected on different days (no more than 1 week apart) because a sampling crew was unable to collect all fish needed to prepare the composite sample on the same day, the individual fish

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should be processed within 24 hours. The fish may have to be filleted and frozen until all the fish to be included in the composite are delivered to the laboratory. At that time, the composite homogenate sample may be prepared.

- *Organisms of the target species should be collected in sufficient numbers to provide sufficient mass of composite homogenate sample of edible tissue for analysis of target analytes:* Composite sample should contain adequate tissue mass so that sufficient material will be available for the analysis of all target analytes. The investigator should determine the required tissue homogenate mass based upon the lowest expected chemical concentration of the target analyte(s) and the sensitivity of the analytical method. Sample mass requirements can vary considerably depending upon the chemical(s) and analytical method. Analyses of PCBs and TCDD/TCDFs in aquatic biota typically requires about 10 to 20 grams (wet weight) of tissue. Up to 200 grams of tissue may be required for multiple analytes (e.g., numerous priority pollutants including PCBs, dioxins, pesticides and metals).

  Given the variability in size among target species, only approximate ranges can be suggested for the number of individual organisms to collect to achieve adequate sample mass (USEPA, 1989a; Versar, 1982). For fish, 3 to 10 individuals should be collected for a composite sample for each target species; for shellfish, 3 to 50 individuals should be collected for a composite sample. In some cases, however, more than 50 small shellfish (e.g., mussels, shrimp, crayfish) may be needed to obtain the recommended 200-g sample mass. The same number of individuals should be used in each composite sample for a given target species at each sampling site.

Deviations from the recommended study design have implications that may make the statistical analyses more complicated. The statistical methods for analyzing composite samples are made tractable and easier-to-use by simplifying the study design. Using equal numbers of organisms in replicate composite samples is one way to do this (USEPA, 2002a).

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For shellfish samples, the recommended composite sample type for chemical analysis should also consider the tissue type consumed by the target population. The specific tissues considered to be edible will vary among target shellfish species based on local consumer preference. For example, several states (Maine, Maryland, Massachusetts, New Hampshire, New Jersey and New York) have issued advisories for a variety of contaminants (PCBs, dioxins/furans, or cadmium) in specific glands or tissues of crustaceans such as lobsters and crabs. Some consumers of lobsters, *Homarus americanus,* enjoy eating the tomalley (digestive gland of the lobster), which has been shown to contain higher concentrations of chemical contaminants than the claw, leg, or tail meat typically consumed by members of the general population. Similarly, for the blue crab, *Callinectes sapidus,* as well as other crab species, the hepatopancreas (digestive gland) is consumed by some individuals; this has also been found to contain higher concentrations of contaminants than claw, leg, or body muscle tissue. A precise description of the sample type (including the number and size of the individual crustaceans in the composite) should be documented in the program record for each target species. A similar situation exists with respect to selection of the appropriate sample type for bivalve molluscs.

For freshwater turtles, the study objectives and sample type consumed by the target population at risk should be of primary consideration. However, EPA recommends use of individual turtle samples rather than composite samples for evaluating turtle tissue contamination. As with shellfish, the tissues of freshwater turtles considered to be edible vary based on the dietary and culinary practices of local populations.

3.3.6 Replicate Samples

EPA recommends that replicate composite samples of each target species be collected at each sampling site. Field replicates are distinct composite samples comprised of tissue from different individuals of the same species. They should be collected at a minimum of 10 percent of the sampling sites (USEPA, 2000b). The collection and storage of replicate samples, even if not analyzed at the time due to inadequate resources, allow for follow up QC checks. These sites should be identified during the planning phase.

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3.3.7 Sample Collection Methods

The selection of equipment and methods for sampling fish and shellfish is a topic beyond the scope of this document. In response to the variations in environmental conditions and target species of interest, fisheries biologists have had to devise sampling methods that are intrinsically selective for certain species and sizes of fish and shellfish (Versar, 1982). This selectivity is a great advantage for sampling biota for the purpose of measuring a BAF, because minimizing factors such as differences in taxa and size improves the accuracy of the measurement.

Sample collection activities can be initiated in the field only after an approved sampling plan has been developed and all permits for collection are in hand. Recommended sampling equipment and its use, considerations for ensuring preservation of sample integrity, and field record keeping and chain-of-custody procedures associated with sample processing, preservation, and shipping are discussed in USEPA (2000b; 1997a).

3.4 MEASURING CHEMICAL CONCENTRATIONS IN WATER

This section provides guidance on the development of a field plan for sampling water in support of the measurement of a site-specific BAF. For water sampling, there are seven major parameters to be specified prior to the initiation of any field water sample collection activities:

- Target analytes and analytical methods
- Phase separation
- Sampling locations
- Sampling times
- Sample type
- Replicate samples
- Sample collection methods

The role of each of these in developing an appropriate field plan for water sampling is discussed below. As was the case for biota sampling, the investigator and field sampling staff

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should develop a detailed sampling plan prior to initiating the field study. In addition to the documents specifically referenced below, EPA publishes a number of guidance documents for environmental sampling design. These include the *Superfund Program Representative Sampling Guidance, Volume 5: Water and Sediment, Part I* (USEPA, 1995) and the EPA Quality System Documents, located at: http://www.epa.gov/quality/qa_docs.html (e.g., *Guidance for Choosing a Sampling Design for Environmental Data Collection, EPA QA/G-5S*; USEPA. 2002a).

3.4.1 Target Analytes and Analytical Methods

The method used to analyze water samples for concentrations of chemicals of concern must be compatible and consistent with the method selected for analysis of biota samples (Section 3.3.1). Bioaccumulation factors are only determined for individual chemicals. In cases where the chemical of interest is a mixture (e.g., PCBs, chlordane), individual chemicals composing the mixture should be quantified individually. This will result in BAFs for individual components of the chemical mixture. Where appropriate, BAFs should be expressed for specific forms of the chemical of concern.

The investigator should also ensure that the method chosen to analyze chemicals of concern in water is sufficiently sensitive to measure ambient concentrations. For highly-bioaccumulative chemicals, sensitivity of the analytical method is commonly more critical for water than it is for biota; for these chemicals the water concentration may be vanishingly small even though the concentrations in tissue are high enough to cause concern. Unless the investigator is confident that chemical concentrations can be measured in the majority (preferably > 80%) of both biota and water samples, a different approach of determining BAFs should be considered. This is why the BSAF approach is preferred for highly-hydrophobic nonionic chemicals that are difficult or impossible to analyze in water.

Contamination of water samples is also an issue of concern, especially when ambient concentrations are very low. The field sampling plan should incorporate adequate blank samples to detect contamination from sampling equipment and containers, as well as contamination that

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can occur during sample transport, handling and analysis. Guidance regarding the use of blanks and other quality control samples is provided in USEPA (2002b).

For organic chemicals with log K_{ow} >4, POC and DOC concentrations should be measured in all water samples along with the chemicals of concern. Measuring POC and DOC will allow the investigator to estimate the freely dissolved chemical fraction using Equation 3-6. Other parameters such as temperature, pH, dissolved oxygen, conductivity/salinity, chlorophyll *a* and total suspended solids can also be measured, as they provide information to help interpret the bioavailability and subsequent bioaccumulation of contaminants by aquatic organisms. pH is an especially important parameter for ionizing organic chemicals (see Section 2.2).

3.4.2 Phase Separation

Calculating the total BAF (Equation 3-1) requires the investigator to measure the total chemical concentration in water. For hydrophobic chemicals, including nonionic organic chemicals, a baseline BAF can be calculated instead (Equation 3-2). The advantage of determining a baseline BAF is that the variability of the BAF will usually be reduced when compared to a total BAF determined at the same site (Burkhard et al. 2003; USEPA, 2003). Normalizing the total BAF also reduces the variance between sites and trophic levels for organic chemicals. However, the investigator needs the freely dissolved chemical concentration in water in order to calculate the baseline BAF. The freely dissolved (or bioavailable) chemical concentration can be calculated from the total chemical concentration measured in water, by calculating the freely dissolved chemical fraction:

$$f_{fd} = 1 / (1 + POC \cdot K_{ow} + 0.08 \cdot DOC \cdot K_{ow}) \qquad \text{(Equation 3-6)}$$

where:

DOC = the average dissolved organic carbon concentration in the water column (kg of organic carbon/L of water) and

POC = the average particulate organic carbon concentration in the water column (kg of organic carbon/kg of particulate matter).

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K_{ow} is used to estimate the partition coefficient between POC and freely dissolved chemical, and 0.08 K_{ow} is used to estimate partition coefficient between DOC and freely dissolved chemical (Burkhard, 2000). The applicability of Equation 3-6 has been demonstrated for many nonionic organic chemicals and in numerous aquatic ecosystems (see Section 4.2 of TSD Volume 2; USEPA, 2003).

Alternatively, dissolved concentrations of hydrophobic chemicals can be measured directly by separating the water sample into different fractions. There are a number of methods of achieving chemical phase separation that have been developed for water sampling including filtration, semi-permeable membrane devices [SPMD], solvent-filled dialysis bags, centrifugation and solid phase extraction. Depending on the method, the investigator may still need to apply Equation 3-6 to determine dissolved bioavailable concentrations, because not all methods will effectively separate DOC-bound chemical from the truly dissolved fraction. The preferred option for calculating the freely dissolved chemical concentration will often be apparent once the investigator considers the feasibility and logistics associated with collecting the necessary volumes of total versus phase-separated water samples.

3.4.3 Sampling Locations

Probably the most important factor in measuring a BAF with predictive power is that the water sampling locations be reflective of the immediate home range of the target organism (Section 3.3.2 and 3.3.3). The importance of collecting water samples which are reflective of the organism's home range can not be overstated. Spending time and resources to better define this relationship will greatly decrease the uncertainty associated with the resulting BAFs.

Once the home range of the target organism has been defined for the site of interest, the investigator can select water sampling locations that best reflect the organism's chemical exposure. In this regard, much of Section 3.3.3 for selection of biota sampling locations is also appropriate for water sampling. Information about the preferences of the target organism - in terms of environmental factors (e.g., temperature, transparency, light penetration, depth, water velocity, substrate type, vegetation cover or debris) - can be valuable in terms of sampling the

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water at the most frequently-inhabited locations. If information is available about concentration gradients in the waterbody, samples should be collected along this gradient. If such data are not available, the investigator can solicit expert opinion as to where concentration gradients should be expected. References such as Chapra and Reckhow (1983) and Thomann and Meuller (1987), that deal with expected chemical concentration gradients for different waterbody types and appropriate sampling designs, can be especially valuable in this regard.

3.4.4 Sampling Times

Temporal as well as spatial variability can be high for water concentrations of certain chemicals. As was shown in Section 3.3.3, concentrations of many hydrophobic chemicals in water are expected to be much more variable than concentrations in aquatic organisms. Thus, individual water samples taken at one point in time will likely not be adequate to reflect average exposure to the target species. Water samples should be collected and analyzed so that chemical concentrations can be averaged over the approximate time it takes for the target species to reach steady state, which varies depending on the hydrophobicity and rate of metabolism of the chemical within the target organism. For example, chemicals with high K_{ow} values are expected to reach steady-state in top trophic level organisms much more slowly than chemicals with low K_{ow} values; thus, they require greater temporal averaging of water column concentrations for estimating BAFs. For large fish, a year or longer may be required for concentrations of highly hydrophobic organic chemicals to reach steady state. Other factors to consider when determining the frequency of sampling include target species migration and other aspects of life history.

The investigator should also consider how concentrations of the chemical of concern respond to the various sources, transport and fate pathways at the site. Generally, temporal variations in chemical concentration are related to factors such as seasonality, flow rate, stratification and external loading and fluxes. Water quality studies and models can be extremely valuable guides for predicting how bioavailable chemical concentrations will vary with time. The investigator can check for their availability at the site of interest or at comparable sites via federal or state programmatic activities (e.g., TMDL, Superfund). Water quality models (site-

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specific or generic) can also be used to test alternative sampling designs, as was demonstrated in Section 3.3.3.

3.4.5 Sample Type

Water samples collected to support the measurement of a site-specific BAF may be analyzed for target chemical concentrations individually or as composites. The objective of measuring the average chemical exposure to the target species can be accomplished by either type of sampling. Compositing may be a better water sampling strategy for highly-hydrophobic chemicals (log K_{ow}>4) than for less hydrophobic chemicals, as discussed in Section 3.3.3.

Compositing can be used to reduce the number of analyses required to determine the average chemical concentration in water. Depending on the specific method, reducing the number of analyses may substantially lower the cost. Composites may be formed from water samples either temporally (i.e., samples collected at different times at one location) or spatially (samples collected at one time at different locations). For temporal composite samples, the analytical method-specific holding time should not be exceeded for any portion of the sample. The investigator should consider whether there is enough information to form a composite that will result in a measured concentration comparable to the average exposure to the organism of interest. If not, the composite sample may produce a biased mean concentration. In this case, individual sample analysis may result in a more accurate average water concentration and BAF.

3.4.6 Replicate Samples

Samples should be replicated in order to evaluate precision of sample collection and analytical methods, regardless of whether individual samples or composites are collected. EPA recommends that replicate water samples be collected for a minimum of 10 percent of water samples, although replication rates of 30% or higher are often used for water sampling in field studies (TAMS, 1998; USEPA, 1989b; USEPA, 2004) . Compositing replicate samples collected at one location and time is an effective way to improve precision.

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3.4.7 Sample Collection Methods

Many different methods have been developed for water sampling, so it is difficult to provide generalized guidance beyond using methods that are appropriate for the chemical of concern. Sampling techniques and equipment tend to be specific to classes of chemicals, often due to unique concerns. A good example is the "clean-technique" protocol developed for low-level trace metal sampling, for which avoiding sample contamination is an overriding objective. For hydrophobic organic chemicals, minimizing the contact of water samples with surfaces and sorptive materials prior to extraction is a priority. For volatile chemicals, eliminating contact with the atmosphere and container head spaces are priorities.

Similarly, water sampling techniques and the associated equipment are often somewhat specialized for different waterbody types. Sampling water from a deep lake calls for methods different from those appropriate for sampling a shallow fast-flowing stream.

There are numerous sources of information that can be used by the investigator to select sample collection methods appropriate for the site and the chemical of concern. *Standard Methods for the Examination of Water and Wastewater* (APHA, 2004) is a reference that covers many aspects of water monitoring, including sample collection. *The American Society for Testing and Materials (ASTM)* also publishes references that address methods for collecting water samples.

Another valuable resource for information about water sample collection methods is the *National Field Manual for the Collection of Water-Quality Data* (USGS, 1998). This manual is a collection of handbooks devoted to subjects including:

- Preparations for Water Sampling
- Selection of Equipment for Water Sampling
- Cleaning of Equipment for Water Sampling
- Collection of Water Samples
- Processing of Water Samples

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The *National Field Manual* addresses sampling of novel chemical contaminants such as wastewater, pharmaceutical, and antibiotic compounds; arsenic species; and low-level mercury.

Another useful source of information for water sampling methods are EPA documents such as Standard Operating Procedures (SOPs) developed for specific field studies. These documents often reflect the state of the art in terms of sampling and analytical methods, which may be more current than the reference materials listed previously. For example, the *Lake Michigan Mass Balance Methods Compendium* (USEPA, 1997) describes the sampling and analytical methods used in that study. The *Methods Compendium* describes SOPs for water sample collection specifically for atrazine and atrazine metabolites, nonionic hydrophobic organic chemicals (PCB congeners and trans-nonachlor), mercury, and particulate and dissolved organic carbon.

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APPENDICES

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Appendix 3A

Determining the Number of Samples to Collect for a BAF Measurement: Bootstrap Analysis

The accuracy of BAF measurements calculated from different numbers of chemical concentration measurements can be estimated by Bootstrap resampling. This common numerical simulation method can be used by the investigator to determine the required numbers of biota and water samples. This method will be demonstrated by examples using Green Bay Mass Balance Study PCB congener data.

Bootstrap sampling method

Bootstrap estimation is a computer intensive resampling method for estimating sampling distributions and confidence limits of statistics for which the theoretical sampling distribution is not known (Efron, 1982). To estimate 90% confidence limits for the mean of n samples, one would repeatedly (i.e., thousands of times) select n values with replacement from the original data and calculate the mean of each bootstrap sample. The 95th and 5th percentiles of the distribution of bootstrap means are estimates of the 90% confidence limits. The bootstrap algorithm can be implemented on a personal computer in a number of ways (Simon, 1997). The advantage of the bootstrap is that the investigator is not required to make any assumptions regarding the distribution of data (Simon, 1969), thereby avoiding potential errors in statistical analysis if these assumptions are violated.

In this case, the investigator is interested in estimating the accuracy[5] of BAFs in terms of precision[6] and bias for alternative numbers of biota (n_b) and water (n_w) samples. The bootstrap is applied to resample n_b biota and n_w water concentrations from a high quality data set. For each resample, mean biota and water concentrations are calculated, and then the BAF is calculated as the ratio of the mean concentrations. This procedure is repeated many times, until a stable distribution of BAF values is generated. The bootstrap distribution of BAFs provide the

[5] Accuracy is the degree of conformity of a measured quantity to its actual (true) value.
[6] Accuracy is the degree of veracity while precision is the degree of reproducibility.

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investigator with estimates of the variance as well as the bias[7]. For example, the 90% confidence limits are estimated by the 95th and 5th percentiles of the bootstrap distribution of BAFs. By repeating this procedure using different numbers of biota (n_b) and water (n_w) samples and comparing the BAF dispersion results, the investigator can determine a sampling design that meets their requirements for BAF accuracy.

Bootstrap estimation *does* require the investigator to have data, which can be a problem. As mentioned previously, EPA is aware of relatively few field data sets that are adequate for evaluating BAF sampling designs. For the present exercise, PCB congener concentration data were combined from zones 3a and 3b of the Green Bay Mass Balance dataset (see Burkhard et al. 2003 and Endicott, 2001 for descriptions of this data). This combination produced a dataset containing measurements of PCB congener concentrations for 93 dissolved water samples, 66 forage fish (alewife and rainbow smelt) samples and 42 predator fish (brown trout and walleye) samples. Concentrations of 4 congeners (BZ[8] 18, 52, 149 and 180), which span the range of hydrophobicity for bioaccumulative PCBs, were selected for BAF calculations. Hawker and Connell (1988) reported the following K_{ow} values for these congeners:

PCB congener	log K_{ow}
18	5.24
52	5.84
149	6.67
180	7.36

None of these congeners are significantly metabolized by aquatic organisms. The PCB concentrations were lipid-normalized and adjusted for bioavailability (i.e., filtered water concentrations were converted to freely dissolved concentrations using equation 3-6) prior to analysis. The concentration data are presented in Appendix 3B.

[7] Bias is the discrepancy of the estimate from the "true" BAF value. In this case, the latter is based on the averages of biota and water concentration data using all available samples. In reality, the sample average is still just an estimate of the true value.

[8] PCB congeners can be identified according to a numbering scheme published by Balschmiter and Zell (1980), commonly referred to as "BZ" numbers.

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Forage and predator fish BAFs were calculated for each of the four PCB congeners. 10,000 bootstrap resamples were used for each calculation. In addition, each calculation was repeated 100 times, to ensure the results were independent of small random fluctuations which were observed between calculations. For each BAF calculation, the following results were saved for analysis:

- The mean BAF and standard deviation;
- the 90 % confidence limits of the BAF distribution;
- the ratio of the 90% BAF confidence limits (upper confidence limit/lower confidence limit) or confidence limit ratio (CLR), which is also proportional to the BAF variance,
- the mean bias, defined below:

$$\text{mean bias} = \left[\sum_{n=1}^{n_b} \left(\text{BAF}_{observed} - \text{BAF}_n \right) \right] / n_b$$

where:

$\text{BAF}_{\text{observed}}$	=	the BAF calculated using all of the observed biota and water concentrations,
BAF_n	=	the BAF calculated using the biota and water concentrations sampled in bootstrap resample n, and
n_b	=	the number of bootstrap resamples used in each calculation (10,000)

and

- the root mean square error (RMSE), defined as:

$$\text{RMSE} = \sqrt{\left[\sum_{n=1}^{n_b} \left(\text{BAF}_{observed} - \text{BAF}_n \right)^2 \right] / n_b}$$

The bootstrap procedure was repeated for sample sizes ranging from 2 to 60 fish, and from 2 to 90 water samples.

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Taylor series approximation

If the investigator makes the assumption that the sampling distributions are approximately normal, then first order Taylor series approximation can also be applied to estimate the variance of the BAF. This provides the investigator with a way to confirm (test) the results of Bootstrap resampling. The Taylor series approximations are developed below for both the total BAF and the baseline BAF. The total BAF is calculated from two measured variables (see equation 3-1): the total concentration of chemical in the appropriate wet tissue of the aquatic organism; and the total concentration of the chemical in the ambient water. The variance of the calculated BAF includes variances each of these variables. The Taylor series approximation for the standard deviation of the total BAF is (Mood et al. 1974):

$$s_{\mathrm{BAF}_T^t} = \frac{1}{\mathrm{C}_w}\left[\left(s_{\mathrm{C}_t}\right)^2 + \left(\mathrm{BAF}_T^t\right)^2 \cdot \left(s_{\mathrm{C}_w}\right)^2 - 2 \cdot r_{tw} \cdot s_{\mathrm{C}_t} \cdot s_{\mathrm{C}_w} \cdot \mathrm{BAF}_T^t\right]^{1/2}$$

where s_{Ct} and s_{Cw} are the standard deviations for C_t and C_w, respectively; and r_{tw} is the correlation coefficient[9] between C_t and C_w. The baseline BAF is calculated from four measured variables (see equation 3-2): the concentration of the chemical in the organism on a wet weight basis; the lipid content of the wet tissue; the total concentration of the chemical in the ambient water; and the fraction of the water concentration that is freely dissolved. The variance of the calculated baseline BAF includes variances each of the four measured variables. For the baseline BAF, the Taylor series approximation for the standard deviation is:

$$s_{\mathrm{BAF}_l^{fd}} = \frac{1}{\mathrm{C}_w^{fd}}\left[\left(s_{\mathrm{C}_l}\right)^2 + \left(\mathrm{BAF}_l^{fd}\right)^2 \cdot \left(s_{\mathrm{C}_w^{fd}}\right)^2 - 2 \cdot r_{ld} \cdot s_{\mathrm{C}_l} \cdot s_{\mathrm{C}_w^{fd}} \cdot \mathrm{BAF}_l^{fd}\right]^{1/2}$$

where:

$$s_{\mathrm{C}_l} = \frac{1}{\mathrm{f}_l}\left[\left(s_{\mathrm{C}_t}\right)^2 + \mathrm{C}_l^{\,2} \cdot \left(s_{f_l}\right)^2 - 2 \cdot r_{lt} \cdot s_{\mathrm{C}_t} \cdot s_{f_l} \cdot \mathrm{C}_l\right]^{1/2}$$

and

[9] We assumed that the PCB concentrations in fish and water were uncorrelated within each of the spatial zones sampled in Green Bay.

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$$s_{C_w^{fd}} = \frac{1}{f_d}\left[\left(s_{C_w}\right)^2 + \left(C_w^{fd}\right)^2 \cdot \left(s_{fd}\right)^2 - 2 \cdot r_{wd} \cdot s_{C_w} \cdot s_{fd} \cdot C_w^{fd}\right]^{1/2}$$

Where s_{BAF_l}, s_{C_l}, and $s_{C_w^{fd}}$ are the standard deviations for the BAF, C_l, and C_w^{fd}, respectively; and r_{ld} is the correlation coefficient[4] between C_l and C_w^{fd}, r_{lt} is the correlation coefficient between C_t and C_l, and r_{wd} is the correlation coefficient between C_w and f_d.

For sufficiently large sample sizes, the approximate (1- /2)·100 percent confidence limits for the BAF (total or baseline) are given by:

$$\overline{\text{BAF}} \pm z_{\alpha/2} \cdot s_{BAF}$$

where is a probability of exceedance and z /2 is the value of the standard normal distribution. Generally, the degree of confidence will only be accurate if the sample size is greater than 30. Taylor series approximation was used to estimate confidence limits for the BAFs in Green Bay. These estimates were then compared with the confidence limits determined using the bootstrap, in order to check and confirm the accuracy of the latter.

Bootstrap BAF results

The distribution of 10,000 chemical concentrations in forage fish and water generated by bootstrap resampling of the Green Bay congener 18 data, as well as the BAFs calculated from the ratio of these concentrations, are displayed as histograms in Figure 3A-1. This particular example was based upon resampling 20 fish and 20 water concentrations. The concentration distributions are symmetrical and approximately normal, as is the distribution of BAFs. If bootstrap resampling is repeated using fewer fish and water concentrations, a number of changes occur in the output distributions of both the chemical concentrations and the BAFs. Figure 3A-2 displays histograms for 2 fish and 20 water concentrations resampled from the same data. The output distribution of fish concentrations is more dispersed (wider) and asymmetrical (the right tail of the distribution is extended) by comparison to Figure 3A-1. This difference is a consequence of resampling fewer (2 as opposed to 20) fish concentrations. As a result, the BAF distribution in Figure 3A-2 is also more dispersed and asymmetrical. A more extreme example of

limiting the sample sizes is displayed in Figure 3A-3, which shows histograms for 2 fish and 2 water concentrations resampled from the same data. The output distributions of both fish and water concentrations are now more dispersed and asymmetrical in comparison to Figure 3A-1, although the effect of reducing the number of concentrations resampled from the data is more pronounced for water than for fish. The BAF distribution in Figure 3A-3 is also more dispersed and asymmetrical than the distributions from the previous examples (Figures 3A-1 and 3A-2). In comparison to Figure 3A-2, the BAF distribution in Figure 3A-3 is notably more asymmetrical. This was an effect on BAF distributions that was observed in all of the bootstrap trials that involved few (less than 6) resamples of water concentrations.

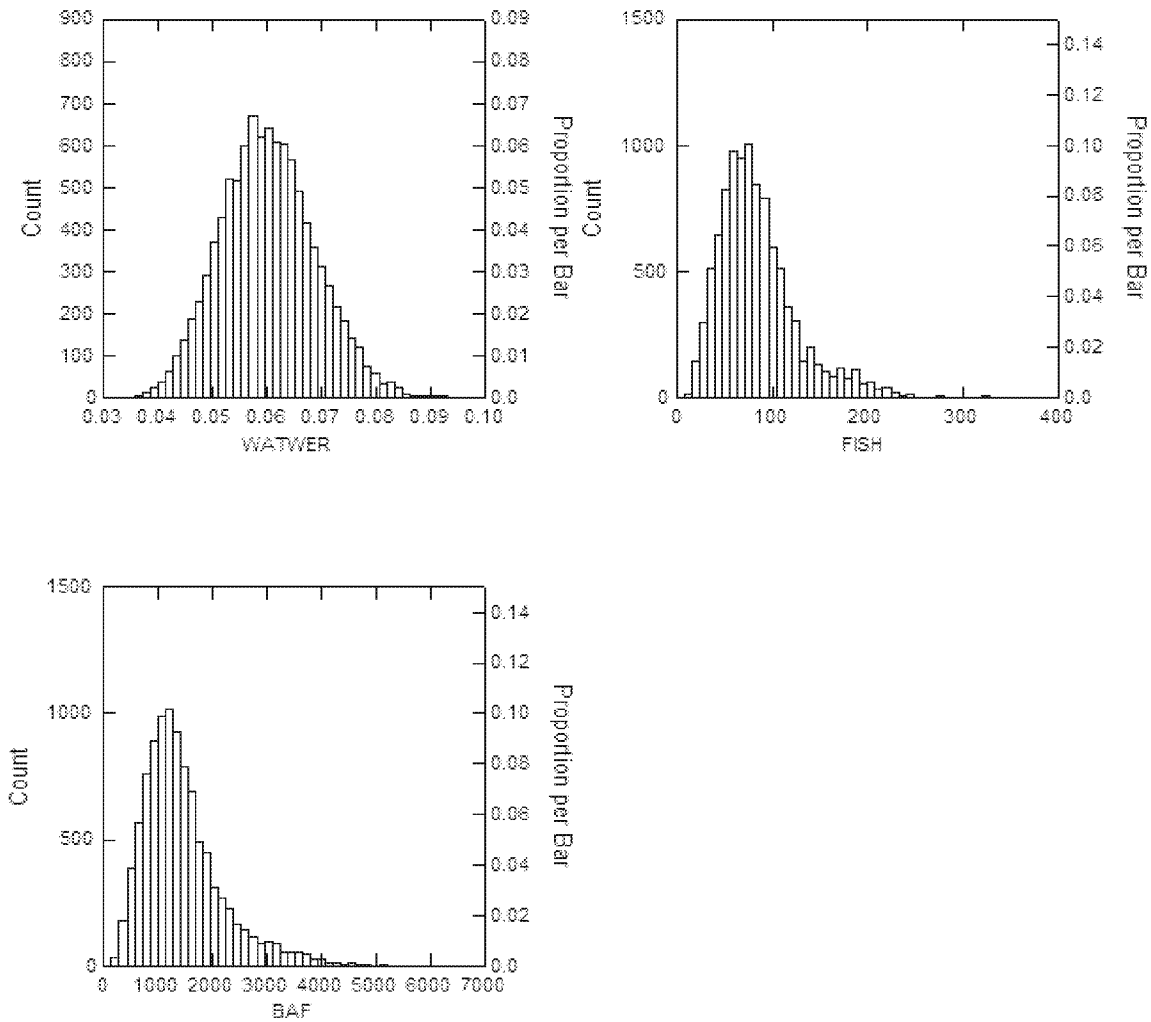

Figure 3A-1. Bootstrap distributions of forage fish and water concentrations, and BAFs based on resampling 20 fish and 20 water concentrations from Green Bay Zone 3 PCB 18 data

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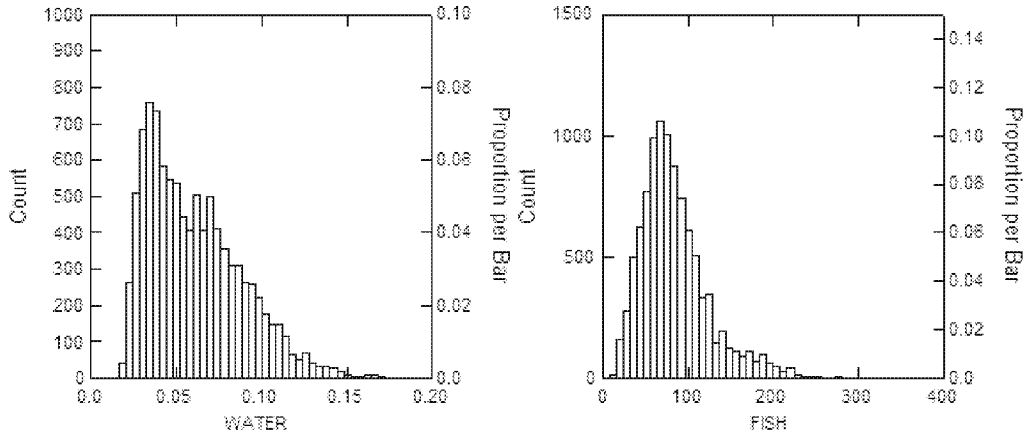

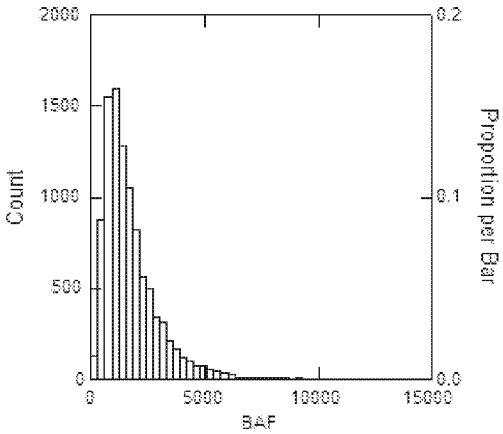

Figure 3A-2. Bootstrap distributions of forage fish and water concentrations, and BAFs based on resampling 2 fish and 20 water concentrations from Green Bay zone 3 PCB 18 data.

Figure 3A-3. Bootstrap distributions of forage fish and water concentrations, and BAFs based on resampling 2 fish and 2 water concentrations from Green Bay zone 3 PCB 18 data.

The 90% confidence limit ratio (CLR) was used as a measure of the precision for the distribution of BAFs in each bootstrap resampling calculation. The CLRs for BAFs determined by resampling varying numbers of fish and water concentrations are tabulated in Table 3A-1 for PCB congener 149 forage fish. The precision of BAF estimates was sensitive to the sample sizes of chemical concentrations in both fish and water, especially for sample sizes smaller than 6. These results also indicate that resampling different combinations of the number of fish and water concentrations can yield comparable BAF precision. For example, the same CLR for PCB

congener 149 forage fish BAF (Table 3A-1) is obtained by resampling 10 fish and 6 water concentrations or 4 fish and 10 water concentrations. The BAF confidence limit ratios are also plotted as functions of resample size in Figure 3A-4. Similar results were obtained for the other congeners (not shown). Predator fish BAF results for each of the congeners were very similar to the results for the corresponding forage fish.

Table 3A-1. Bootstrap Results for PCB Congener 149 Forage Fish BAF: 90% Confidence Limit Ratio (Upper Confidence Limit/Lower Confidence Limit) as a Function of the Number of Fish and Water Samples. Smaller Ratios Indicate Less Uncertainty.

Number of Fish Samples	Number of Water Samples								
	2	**4**	**6**	**8**	**10**	**20**	**30**	**60**	**90**
2	6.65	4.95	4.37	4.04	3.82	3.31	3.13	2.94	2.87
4	5.75	4.10	3.55	3.25	3.06	2.59	2.41	2.23	2.16
6	5.46	3.81	3.29	3.01	2.81	2.35	2.18	1.99	1.92
8	5.32	3.65	3.16	2.87	2.69	2.24	2.06	1.86	1.79
10	5.23	3.55	3.07	2.80	2.61	2.17	1.98	1.78	1.71
20	5.02	3.34	2.91	2.64	2.46	2.02	1.84	1.62	1.55
30	4.95	3.25	2.86	2.59	2.41	1.98	1.79	1.57	1.49
60	4.89	3.17	2.82	2.54	2.35	1.93	1.73	1.51	1.42

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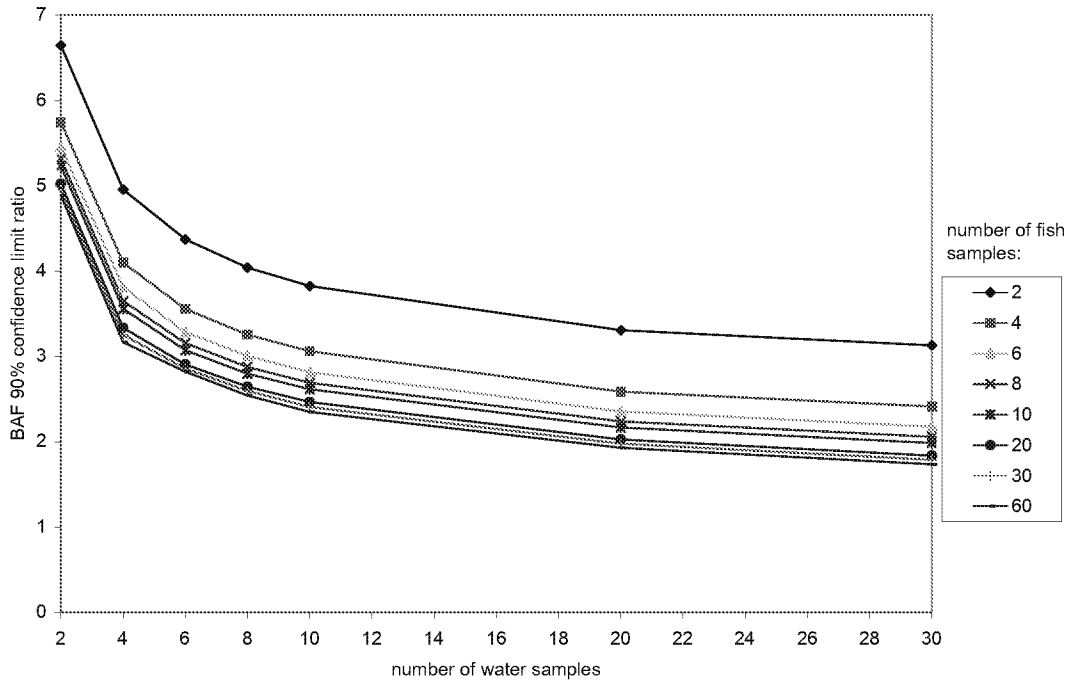

Figure 3A-4. Bootstrap resampling results for PCB congener 149 in Green Bay Zone 3 **forage fish: 90 % confidence limit ratios for BAF as a function of the numbers of** fish and water samples.

Precision is an important component of BAF accuracy, but the investigator should also be concerned with the bias of the BAF measurement. The bootstrap resampling results also demonstrate how bias of the BAF measurement is influenced by resampling different numbers of fish and water concentrations. While the precision of BAFs depended on the sample sizes of both fish and water, the results reveal that the mean bias is only sensitive to the number of water concentrations resampled. This result was somewhat surprising, and appears to be related to the strong influence of variability in the denominator of a ratio. Figure 3A-5 plots the mean percent bias for each of the PCB congener forage fish BAFs as functions of resample size. For each congener, the curves for different numbers of fish concentration resamples fall on top of each other, demonstrating that the mean bias of BAFs is only sensitive to the number of water concentrations resampled. BAF biases for each congener in Figure 3A-5 show the same pattern, depending solely on the sample size of chemical concentrations in water. The bias in BAFs

appears to be correlated to the hydrophobicity of the chemical, as the bias increases slightly for the more hydrophobic PCB congeners.

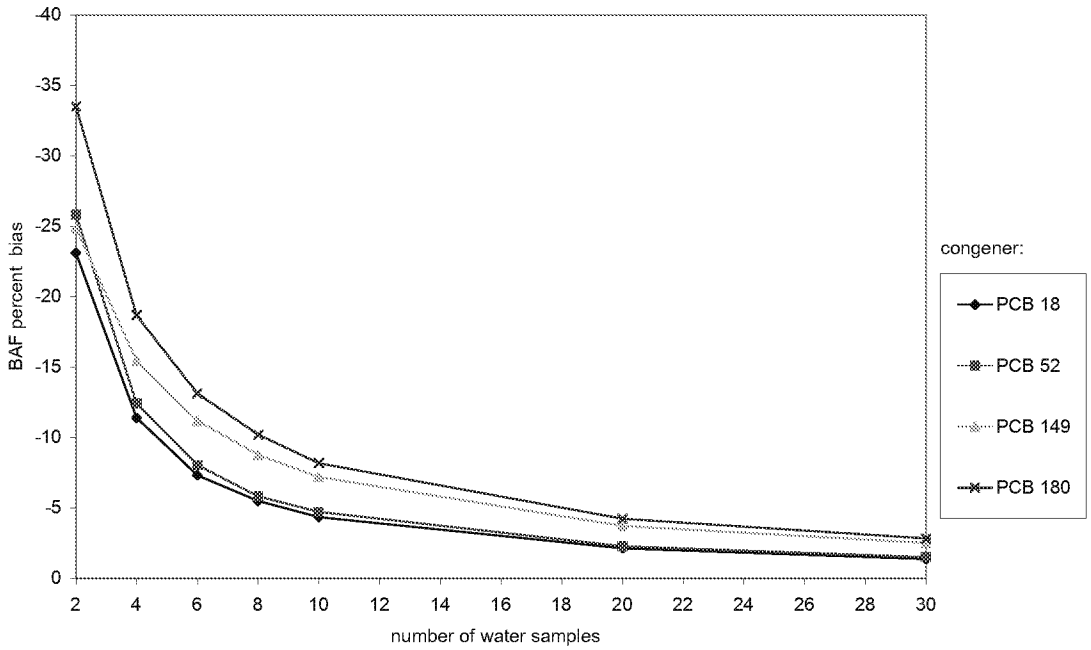

Figure 3A-5. Bootstrap resampling results for PCB congeners 18, 52, 149 and 180 in Green Bay Zone 3 forage fish: Comparison of mean percent bias of BAF as a function of the numbers of water samples.

The root mean square error (RMSE) of the BAFs was also calculated for each bootstrap resample. RMSE is an aggregate measure of accuracy, incorporating both precision and bias. Figure 3A-6 plots the forage fish BAF RMSEs for congener 149 as functions of resample size. The curves plotted in this figure are almost identical in shape to the ratios of BAF confidence limits plotted in Figure 3A-4 . The similarity between the plots of RMSEs and ratios of BAF confidence limits demonstrates that precision is the major component of BAF accuracy in the bootstrap resamples. Therefore, it is reasonable for the investigator to select the appropriate number of biota and water samples based upon the ratios of BAF confidence limits, although the negative mean percent bias associated with sampling fewer than 6 water concentrations should also be considered.

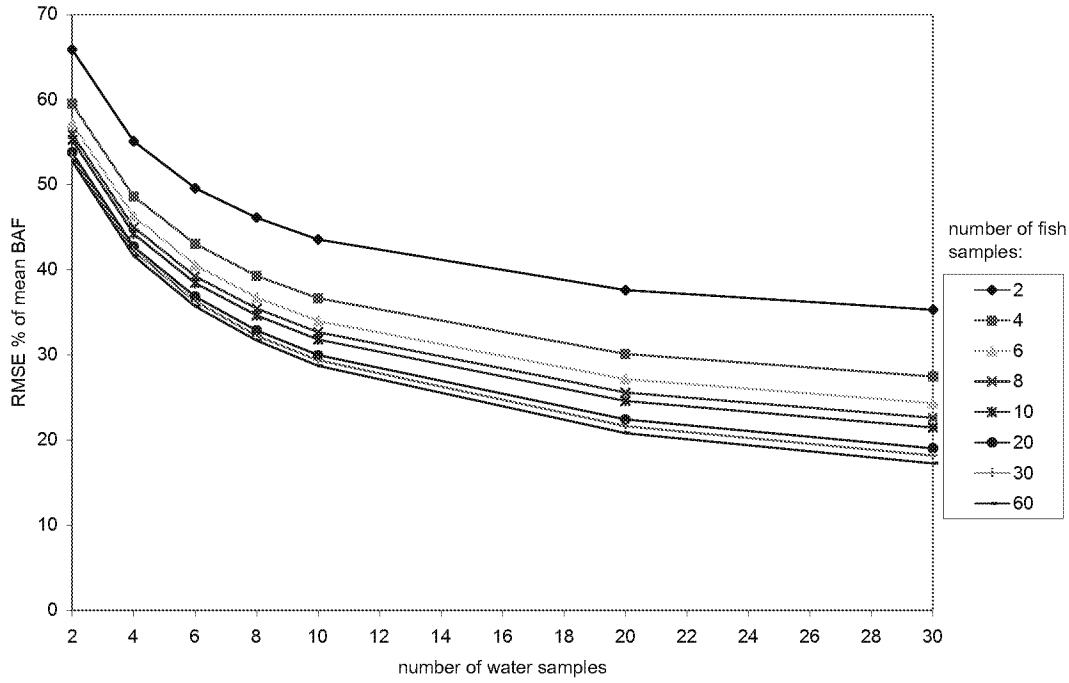

Figure 3A-6. Bootstrap resampling results for PCB congener 149 in Green Bay Zone 3 forage fish: Root mean square error (RMSE) for BAF as a function of the numbers of fish and water samples.

Taylor estimates confirm Bootstrap results

Table 3A-2 presents the concentration statistics (average and standard deviation) for each PCB congener, the statistics for the predator and forage fish BAFs, and the confidence limits and confidence limit ratios calculated using Taylor series approximation. In all cases, the Taylor series confidence limits are practically identical to the confidence limits obtained by bootstrap resampling a large number of fish (n_b) and water (n_w) concentrations. This agreement confirms that the Bootstrap algorithm was performing properly.

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How does compositing affect the relationship between number of samples and the accuracy of the BAF?

Compositing of fish and/or water samples for analysis is recommended as a highly effective option in Sections 3.3.5 and 3.4.5. A single measured concentration from a well-formed composite should be equivalent to the average or mean of concentrations measured in samples used to form the composite. A number of the Bootstrap resampling tests were modified to simulate sample compositing, and the results (e.g., fish and water concentration means, BAFs and 90% CLRs) were compared to the original (unmodified) tests. In all cases, we found no difference between the tests simulating sample compositing versus those that did not. The simulation results indicate that the accuracy of a BAF depends on the number of biota and water samples that are collected, and not on whether the samples are analyzed individually or as composites.

How can the Bootstrap help determine the number of samples to collect and analyze?

If site-specific data for concentrations of the target chemical in biota and water are available, then Bootstrap resampling is probably the best way to determine the number of samples to collect and analyze in order to determine a BAF of the desired accuracy. Bootstrap resampling can also be useful if a site-specific BAF is measured and the uncertainty is found to be unacceptably large. In this case, the bootstrap can be used to estimate the additional sampling effort, in terms of numbers of biota and/or water samples, required to improve the accuracy of the BAF derived from these measurements. Bootstrap resampling is a much less useful tool when site-specific data for concentrations of the target chemical in biota and water are not available. If the investigator can find chemical concentration data for an ecosystem comparable to their site, then bootstrap resampling could be conducted with that data, and sample sizes selected accordingly. However, there are other approaches that should also be considered when site-specific data are limited.

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Table 3A-2. Taylor Series Approximation of Confidence Limits for Baseline BAF$_i$s in Green Bay Zone 3

CHEMICAL AND STATISTIC	CONCENTRATION			PREDATOR FISH				FORAGE FISH			
	Predator Fish C_ℓ (ng/g-ℓ)	Forage Fish C_ℓ (ng/g-ℓ)	Water C_w^{fd} (ng/L)	Baseline BAF_4	90% LCL[a]	90% UCL[b]	90% CLR[c]	Baseline BAF_3	90% LCL	90% UCL	90% CLR
PCB 18:											
Average	183	84.0	0.0601	3,051	1,970	4,132	2.10	1,399	1,151	1,647	1.43
St. deviation	243	57.5	0.0388								
St. error				657				151			
PCB 52:											
Average	1401	649	0.0590	23,760	18,928	28,592	1.51	11,009	9,317	12,700	1.36
St. deviation	931	331	0.0393								
St. error				2,938				1,028			
PCB 149:											
Average	537	240	0.00659	81,502	63,924	99,081	1.55	36,349	30,053	42,645	1.42
St. deviation	329	103	0.00579								
St. error				10,686				3,827			
PCB 180:											
Average	544	211	0.00102	534,098	412,216	655,979	1.59	207,284	168,325	246,242	1.46
St. deviation	350	104	0.000949								
St. error				74,092				23,683			

[a] LCL: Lower confidence limit; [b] UCL: Upper confidence limit; [c] CLR: Confidence limit ratio.

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Appendix 3B

PCB Congener Concentrations Measured by Green Bay Mass Balance Study

1. Green Bay Mass Balance PCB Congener Concentrations: Dissolved Water Column

Date	Zone	Station	Layer[a]	PCB 149 (ng/L)	PCB 18 (ng/L)	PCB 180 (ng/L)	PCB 52 (ng/L)
5/1/1989	GB0Z3A	GB0019		0.0038	0.0165	0.00045	0.0233
5/2/1989	GB0Z3B	GB0018		0.0097	0.0487	0.00070	0.0416
5/3/1989	GB0Z3A	GB0013		0.0047	0.0190	0.00098	0.0346
5/3/1989	GB0Z3A	GB0013		0.0131	0.0578	0.00599	0.0935
5/3/1989	GB0Z3A	GB0014		0.0067	0.0551	0.00088	0.0617
5/3/1989	GB0Z3B	GB0015		0.0037	0.0897	0.00080	0.0817
5/3/1989	GB0Z3A	GB0016		0.0108	0.0294	0.00332	0.0418
5/3/1989	GB0Z3B	GB0017		0.0074	0.1026	0.00089	0.0979
5/4/1989	GB0Z3A	GB0008		0.0061	0.0797	0.00074	0.0716
5/4/1989	GB0Z3A	GB0009		0.0065	0.0472	0.00065	0.0426
5/4/1989	GB0Z3A	GB0010		0.0046	0.0764	0.00051	0.0719
5/4/1989	GB0Z3B	GB0011		0.0052	0.1074	0.00049	0.0941
6/11/1989	GB0Z3A	GB0016		0.0075	0.0217	0.00094	0.0191
6/11/1989	GB0Z3B	GB0017		0.0047	0.0828	0.00059	0.0708
6/11/1989	GB0Z3B	GB0018		0.0046	0.0277	0.00047	0.0244
6/11/1989	GB0Z3A	GB0019		0.0047	0.0204	0.00041	0.0140
6/11/1989	GB0Z3B	GB0020		0.0035	0.0233	0.00052	0.0185
6/12/1989	GB0Z3A	GB0008		0.0057	0.0709	0.00068	0.0674
6/12/1989	GB0Z3A	GB0009		0.0044	0.0459	0.00059	0.0486
6/12/1989	GB0Z3A	GB0010		0.0058	0.0795	0.00088	0.0767
6/12/1989	GB0Z3B	GB0011		0.0078	0.1040	0.00102	0.0974
6/12/1989	GB0Z3A	GB0013		0.0062	0.0374	0.00079	0.0415
6/12/1989	GB0Z3A	GB0013		0.0061	0.0376	0.00080	0.0399
6/12/1989	GB0Z3A	GB0014		0.0041	0.0369	0.00048	0.0374
6/12/1989	GB0Z3B	GB0015		0.0044	0.1018	0.00066	0.0901
7/30/1989	GB0Z3A	GB0016		0.0046	0.0248	0.00074	0.0251
7/30/1989	GB0Z3B	GB0017		0.0038	0.0470	0.00069	0.0499
7/30/1989	GB0Z3B	GB0018	EPI	0.0037	0.0239	0.00025	0.0197
7/30/1989	GB0Z3B	GB0018	HYP	0.0050	0.0282	0.00104	0.0384
7/30/1989	GB0Z3A	GB0019		0.0050	0.0244	0.00060	0.0216
7/30/1989	GB0Z3B	GB0020		0.0037	0.0290	0.00055	0.0309
7/30/1989	GB0Z3B	GB0020		0.0033	0.0240	0.00050	0.0291
7/31/1989	GB0Z3A	GB0008		0.0054	0.1248	0.00081	0.1055
7/31/1989	GB0Z3A	GB0008		0.0072	0.1811	0.00089	0.1817
7/31/1989	GB0Z3A	GB0009		0.0048	0.0536	0.00048	0.0614
7/31/1989	GB0Z3A	GB0010		0.0064	0.1477	0.00072	0.1468
7/31/1989	GB0Z3A	GB0010		0.0061	0.1442	0.00081	0.1455
7/31/1989	GB0Z3B	GB0011		0.0067	0.1395	0.00090	0.1505
7/31/1989	GB0Z3A	GB0013		0.0040	0.0360	0.00056	0.0597
7/31/1989	GB0Z3A	GB0014		0.0035	0.0478	0.00063	0.0528
7/31/1989	GB0Z3B	GB0015		0.0056	0.0333	0.00072	0.0352
9/15/1989	GB0Z3B	GB0018	EPI	0.0030	0.0188	0.00006	0.0145
9/15/1989	GB0Z3B	GB0018	HYP	0.0071	0.0337	0.00107	0.0263
9/15/1989	GB0Z3A	GB0019	EPI	0.0023	0.0204	0.00033	0.0135
9/15/1989	GB0Z3A	GB0019	HYP	0.0039	0.0243	0.00052	0.0278
9/15/1989	GB0Z3B	GB0020		0.0035	0.0211	0.00071	0.0158
9/16/1989	GB0Z3A	GB0009		0.0087	0.1206	0.00094	0.0840
9/16/1989	GB0Z3A	GB0013		0.0053	0.0573	0.00074	0.0392

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Date	Zone	Station	Layer[a]	PCB 149 (ng/L)	PCB 18 (ng/L)	PCB 180 (ng/L)	PCB 52 (ng/L)
9/16/1989	GB0Z3A	GB0013		0.0058	0.0627	0.00117	0.0414
9/16/1989	GB0Z3A	GB0014		0.0055	0.0507	0.00078	0.0387
9/16/1989	GB0Z3B	GB0015	EPI	0.0039	0.0336	0.00091	0.0260
9/16/1989	GB0Z3B	GB0015	HYP	0.0044	0.0432	0.00108	0.0366
9/16/1989	GB0Z3A	GB0016	EPI	0.0040	0.0293	0.00066	0.0215
9/16/1989	GB0Z3A	GB0016	HYP	0.0057	0.0412	0.00108	0.0313
9/16/1989	GB0Z3B	GB0017	EPI	0.0035	0.0267	0.00063	0.0220
9/16/1989	GB0Z3B	GB0017	HYP	0.0038	0.0263	0.00072	0.0219
9/17/1989	GB0Z3A	GB0008		0.0064	0.1256	0.00091	0.1105
9/17/1989	GB0Z3A	GB0010		0.0053	0.0910	0.00079	0.0704
9/17/1989	GB0Z3B	GB0011		0.0061	0.1034	0.00084	0.0932
10/22/1989	GB0Z3A	GB0016		0.0072	0.0386	0.00132	0.0360
10/22/1989	GB0Z3B	GB0018		0.0041	0.0503	0.00112	0.0485
10/22/1989	GB0Z3A	GB0019		0.0061	0.0402	0.00163	0.0380
10/22/1989	GB0Z3B	GB0020		0.0057	0.0297	0.00133	0.0238
10/23/1989	GB0Z3A	GB0009		0.0029	0.0321	0.00092	0.0436
10/23/1989	GB0Z3A	GB0010		0.0046	0.1067	0.00111	0.1312
10/23/1989	GB0Z3B	GB0011		0.0047	0.1153	0.00125	0.1246
10/23/1989	GB0Z3A	GB0013		0.0044	0.0452	0.00143	0.0518
10/23/1989	GB0Z3A	GB0013		0.0095	0.0506	0.00123	0.0548
10/23/1989	GB0Z3A	GB0014		0.0065	0.1133	0.00126	0.1132
10/23/1989	GB0Z3B	GB0015		0.0043	0.0927	0.00099	0.0961
10/23/1989	GB0Z3B	GB0017		0.0038	0.0429	0.00100	0.0459
10/24/1989	GB0Z3A	GB0008		0.0052	0.1508	0.00085	0.1543
2/8/1990	GB0Z3B	GB0015		0.0229	0.0673	0.00257	0.0948
2/9/1990	GB0Z3A	GB0013		0.0266	0.0512	0.00395	0.0725
2/9/1990	GB0Z3A	GB0014		0.0274	0.0713	0.00340	0.0994
2/10/1990	GB0Z3A	GB0009		0.0267	0.0785	0.00379	0.0891
2/10/1990	GB0Z3A	GB0010		0.0256	0.1139	0.00378	0.1360
2/10/1990	GB0Z3B	GB0011		0.0307	0.1613	0.00322	0.1400
2/11/1990	GB0Z3A	GB0008		0.0179	0.1048	0.00233	0.1148
4/28/1990	GB0Z3A	GB0016		0.0034	0.0321	0.00039	0.0280
4/28/1990	GB0Z3B	GB0017		0.0044	0.0590	0.00044	0.0493
4/28/1990	GB0Z3B	GB0018		0.0027	0.0233	0.00039	0.0216
4/28/1990	GB0Z3A	GB0019		0.0045	0.0205	0.00089	0.0175
4/28/1990	GB0Z3B	GB0020		0.0051	0.0247	0.00006	0.0201
4/29/1990	GB0Z3A	GB0008		0.0033	0.0733	0.00077	0.0618
4/29/1990	GB0Z3A	GB0009		0.0033	0.0415	0.00037	0.0330
4/29/1990	GB0Z3A	GB0010		0.0037	0.0331	0.00040	0.0293
4/29/1990	GB0Z3A	GB0010		0.0025	0.0339	0.00064	0.0241
4/29/1990	GB0Z3B	GB0011		0.0025	0.0479	0.00023	0.0412
4/29/1990	GB0Z3A	GB0013		0.0029	0.0389	0.00036	0.0326
4/29/1990	GB0Z3A	GB0014		0.0031	0.0369	0.00028	0.0340
4/29/1990	GB0Z3A	GB0014		0.0029	0.0355	0.00028	0.0344
4/29/1990	GB0Z3B	GB0015		0.0038	0.0699	0.00063	0.0581

[a] Layer refers to epilimnion (EPI) and hypolimnion (HYP) sampled separately when the water column was thermally stratified; otherwise, a single mid-depth sample was collected.

2. Green Bay Mass Balance PCB Congener Concentrations: Lipid-Normalized Forage Fish

Common Name	Life Stage[b]	Zone	Date	PCB 149 (ng/g-l)	PCB 18 (ng/g-l)	PCB 180 (ng/g-l)	PCB 52 (ng/g-l)
ALEWIFE	A	GB0Z3A	6/10/1989	288	13	240	367
ALEWIFE	A	GB0Z3A	6/10/1989	454	91	522	295
ALEWIFE	A	GB0Z3A	6/10/1989	513	37	587	611
ALEWIFE	A	GB0Z3A	10/3/1989	197	132	184	724
ALEWIFE	A	GB0Z3A	6/10/1989	352	110	288	1072
ALEWIFE	A	GB0Z3A	8/8/1989	402	44	366	512
ALEWIFE	A	GB0Z3A	10/3/1989	197	68	197	518
ALEWIFE	A	GB0Z3A	10/3/1989	258	53	258	549
ALEWIFE	A	GB0Z3A	8/8/1989	292	36	292	551
ALEWIFE	A	GB0Z3A	6/10/1989	326	33	274	223
ALEWIFE	A	GB0Z3B	6/5/1989	326	42	357	403
ALEWIFE	A	GB0Z3B	10/4/1989	206	266	188	786
ALEWIFE	A	GB0Z3B	10/4/1989	229	165	182	882
ALEWIFE	A	GB0Z3B	10/4/1989	240	146	218	874
ALEWIFE	A	GB0Z3B	8/22/1989	465	323	374	1200
ALEWIFE	A	GB0Z3B	9/13/1989	157	99	157	619
ALEWIFE	A	GB0Z3B	9/13/1989	188	139	172	694
ALEWIFE	Y	GB0Z3A	6/10/1989	311	54	199	466
ALEWIFE	Y	GB0Z3A	6/16/1989	275	25	213	296
ALEWIFE	Y	GB0Z3A	10/3/1989	88	29	64	201
ALEWIFE	Y	GB0Z3A	6/20/1989	195	47	129	570
ALEWIFE	Y	GB0Z3A	6/16/1989	286	49	245	495
ALEWIFE	Y	GB0Z3A	9/12/1989	58	36	43	228
ALEWIFE	Y	GB0Z3A	9/12/1989	137	232	87	847
ALEWIFE	Y	GB0Z3A	9/12/1989	40	22	32	134
RAINBOW SMELT	A	GB0Z3A	10/3/1989	232	79	232	732
RAINBOW SMELT	A	GB0Z3A	6/16/1989	152	23	159	184
RAINBOW SMELT	A	GB0Z3A	5/17/1989	479	115	479	1310
RAINBOW SMELT	A	GB0Z3A	5/1/1989	235	54	220	568
RAINBOW SMELT	A	GB0Z3A	6/16/1989	171	63	133	255
RAINBOW SMELT	A	GB0Z3A	8/9/1989	192	73	167	627
RAINBOW SMELT	A	GB0Z3A	8/9/1989	223	117	223	881
RAINBOW SMELT	A	GB0Z3A	8/9/1989	229	69	229	758
RAINBOW SMELT	A	GB0Z3A	10/3/1989	298	141	215	1242
RAINBOW SMELT	A	GB0Z3A	10/3/1989	204	80	172	768
RAINBOW SMELT	A	GB0Z3A	5/17/1989	376	97	279	940
RAINBOW SMELT	A	GB0Z3B	5/1/1989	304	103	234	1125
RAINBOW SMELT	A	GB0Z3B	5/1/1989	325	172	240	1266
RAINBOW SMELT	A	GB0Z3B	10/4/1989	184	64	184	485
RAINBOW SMELT	A	GB0Z3B	5/1/1989	440	101	377	1289
RAINBOW SMELT	A	GB0Z3B	5/1/1989	387	113	327	1488
RAINBOW SMELT	A	GB0Z3B	5/1/1989	427	140	305	1463
RAINBOW SMELT	A	GB0Z3B	8/9/1989	235	112	199	866
RAINBOW SMELT	A	GB0Z3B	10/4/1989	234	79	249	685
RAINBOW SMELT	A	GB0Z3B	8/9/1989	231	121	190	883
RAINBOW SMELT	A	GB0Z3B	10/4/1989	267	103	200	867
RAINBOW SMELT	A	GB0Z3B	8/9/1989	195	97	154	832
RAINBOW SMELT	Y	GB0Z3A	6/16/1989	135	30	111	175
RAINBOW SMELT	Y	GB0Z3A	6/20/1989	100	21	84	197
RAINBOW SMELT	Y	GB0Z3A	6/16/1989	103	11	79	165
RAINBOW SMELT	Y	GB0Z3A	6/20/1989	125	24	89	204
RAINBOW SMELT	Y	GB0Z3A	9/14/1989	187	43	199	474
RAINBOW SMELT	Y	GB0Z3A	8/9/1989	162	48	157	392
RAINBOW SMELT	Y	GB0Z3A	10/3/1989	239	59	256	657
RAINBOW SMELT	Y	GB0Z3A	9/14/1989	198	23	198	455
RAINBOW SMELT	Y	GB0Z3A	10/3/1989	160	45	135	451

Common Name	Life Stage[b]	Zone	Date	PCB 149 (ng/g-l)	PCB 18 (ng/g-l)	PCB 180 (ng/g-l)	PCB 52 (ng/g-l)
RAINBOW SMELT	Y	GB0Z3A	10/3/1989	155	83	179	361
RAINBOW SMELT	Y	GB0Z3B	5/1/1989	161	109	100	697
RAINBOW SMELT	Y	GB0Z3B	5/1/1989	169	113	96	733
RAINBOW SMELT	Y	GB0Z3B	5/1/1989	143	77	101	568
RAINBOW SMELT	Y	GB0Z3B	11/2/1989	156	78	147	459
RAINBOW SMELT	Y	GB0Z3B	9/5/1989	235	67	216	603
RAINBOW SMELT	Y	GB0Z3B	8/9/1989	198	66	144	519
RAINBOW SMELT	Y	GB0Z3B	11/2/1989	193	66	162	761
RAINBOW SMELT	Y	GB0Z3B	8/9/1989	272	91	211	603
RAINBOW SMELT	Y	GB0Z3B	11/2/1989	225	87	225	734

[b] Forage fish were composited as adult (A) and young-of-year (Y).

3. Green Bay Mass Balance PCB Congener Concentrations: Lipid-Normalized Predator Fish

Common Name	Age	Zone	Date	PCB 149 (ng/g-l)	PCB 18 (ng/g-l)	PCB 180 (ng/g-l)	PCB 52 (ng/g-l)
BROWN TROUT	2	GB0Z3A	9/26/1989	380	101	397	930
BROWN TROUT	2	GB0Z3A	6/9/1989	251	120	215	902
BROWN TROUT	2	GB0Z3A	6/9/1989	269	72	239	595
BROWN TROUT	2	GB0Z3A	9/11/1989	523	133	413	1253
BROWN TROUT	2	GB0Z3A	7/30/1989	298	169	264	1220
BROWN TROUT	2	GB0Z3A	7/22/1989	531	72	493	830
BROWN TROUT	2	GB0Z3A	9/26/1989	120	391	80	879
BROWN TROUT	2	GB0Z3B	10/10/1989	618	163	618	1410
BROWN TROUT	2	GB0Z3B	6/17/1989	252	35	229	387
BROWN TROUT	2	GB0Z3B	8/22/1989	730	1265	652	3891
BROWN TROUT	2	GB0Z3B	10/10/1989	775	164	760	1639
BROWN TROUT	2	GB0Z3B	9/9/1989	412	79	461	889
BROWN TROUT	2	GB0Z3B	10/10/1989	657	144	657	1540
BROWN TROUT	3	GB0Z3A	9/26/1989	541	123	484	1233
BROWN TROUT	3	GB0Z3A	6/6/1989	531	116	558	1157
BROWN TROUT	3	GB0Z3A	9/11/1989	561	89	507	1095
BROWN TROUT	3	GB0Z3A	7/22/1989	363	85	357	907
BROWN TROUT	3	GB0Z3A	9/26/1989	884	198	976	2375
BROWN TROUT	3	GB0Z3A	9/11/1989	386	95	332	813
BROWN TROUT	3	GB0Z3B	10/10/1989	467	71	484	710
BROWN TROUT	3	GB0Z3B	10/18/1989	480	69	516	720
BROWN TROUT	3	GB0Z3B	10/18/1989	667	91	694	1129
BROWN TROUT	3	GB0Z3B	5/17/1989	369	141	336	1142
BROWN TROUT	3	GB0Z3B	9/12/1989	579	113	632	1129
BROWN TROUT	3	GB0Z3B	9/8/1989	466	76	519	916
WALLEYE	3	GB0Z3B	10/22/1989	115	278	431	2011
WALLEYE	3	GB0Z3B	5/11/1989	444	102	389	1019
WALLEYE	3	GB0Z3B	10/21/1989	603	86	619	1253
WALLEYE	3	GB0Z3B	5/2/1989	587	147	587	1602
WALLEYE	3	GB0Z3B	7/12/1989	544	97	575	1109
WALLEYE	3	GB0Z3B	8/22/1989	545	79	529	1184
WALLEYE	4	GB0Z3A	6/19/1989	563	188	563	1578
WALLEYE	4	GB0Z3A	11/9/1989	141	66	562	1171
WALLEYE	4	GB0Z3A	9/11/1989	333	81	360	752
WALLEYE	4	GB0Z3A	11/9/1989	620	1089	544	3183
WALLEYE	4	GB0Z3A	8/31/1989	439	69	439	986
WALLEYE	4	GB0Z3B	5/2/1989	560	93	412	994
WALLEYE	4	GB0Z3B	10/21/1989	738	114	631	1342
WALLEYE	4	GB0Z3B	7/12/1989	701	116	701	1590
WALLEYE	4	GB0Z3B	7/12/1989	660	156	546	1921
WALLEYE	4	GB0Z3B	10/22/1989	2292	542	2500	5625
WALLEYE	4	GB0Z3B	5/2/1989	568	220	568	1833

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Appendix 3C

Determining the Number of Samples to Collect for a BAF Measurement: Monte Carlo Analysis

To demonstrate the Monte Carlo method, a Latin Hypercube Monte Carlo generator program (Wyss and Jorgensen, 1998) was used to simulate 300 chemical concentrations in biota and water. Based upon previous statistical analysis of the Green Bay PCB congener data (Endicott, 2001), we assumed that chemical concentrations were lognormally distributed. Means and variances were calculated from the log-transformed concentration data. The Latin Hypercube method guarantees that the simulated probability distributions of the concentrations are exact, which is advantageous because it reduces the number of realizations necessary for the results to converge (McKay et al. 1979). The generator program also applied Iman and Conover's (1982) algorithm for inducing the specified degree of rank correlation between chemical concentrations in biota and water, without altering their statistical distributions. Statistical properties of all parameter distributions output by the Latin Hypercube generator were confirmed against the specified variance-covariance structure in each test.

The Monte Carlo analysis was based on the same data used in the Bootstrap resampling method (Green Bay Mass Balance data for PCB congeners 18, 52, 149 and 180 in zone 3) so that the results of the two methods could be directly compared. In practice, the investigator would be faced with the problem of estimating appropriate concentration distributions without site-specific data. When such prior information about chemical concentrations is lacking, the investigator has a limited number of choices:

- Conduct a pilot study - a small number of samples may provide suitable preliminary estimate of the concentration distribution;
- Use data from a study of a similar site; or
- Use a crude approximation of standard deviation adopted from USEPA (1989a): Given a range of concentrations (based on judgment or expert opinion), an approximate value of standard deviation may be computed by dividing the concentration range by 6.

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A variety of tests were also run to see how sensitive the results of Monte Carlo analysis were to the following factors:

- Different degrees of correlation between biota and water chemical concentrations,
- Different variances in chemical concentrations, and
- Inappropriate choice of concentration distributions.

How do the results of Monte Carlo and Bootstrap analyses compare?

In general, the analysis of BAF accuracy as a function of sample sizes using the Monte Carlo method produced results comparable to those obtained via Bootstrap resampling. The Monte Carlo results for BAF confidence limit ratios were less sensitive to sample sizes than the Bootstrap results, as shown in Figure 3C-1. The Monte Carlo results also tended to underestimate the bias in BAF ratios. The two methods produced quite similar values of RMSE, except for large sample numbers, when the Monte Carlo RMSE estimates were smaller. Overall, the Monte Carlo BAF statistics are consistent with the Bootstrap results. This outcome is expected, given that the concentration distributions used in the Monte Carlo method were based on the same data used in the Bootstrap resampling. For this case, when the information regarding chemical concentrations are input consistently, both methods function properly and generate comparable results.

The Monte Carlo and Bootstrap predictions of BAF confidence limit ratios for congener 149 forage fish are compared in Figure 3C-2. As was the case for predator fish, the two methods produce generally comparable results for this congener, as well as the others (not shown). The Monte Carlo results were again less sensitive to sample sizes than the Bootstrap results. The concentration data for forage fish were normally distributed, although a lognormal distribution was assumed in the Monte Carlo simulation. Apparently, mis-specifying the concentration distribution had little or no effect on the outcome of the Monte Carlo analysis. This result is consistent with the guidance offered by Berthouex and Brown (1994), that making the lognormal assumption was usually beneficial or (at worst) harmless.

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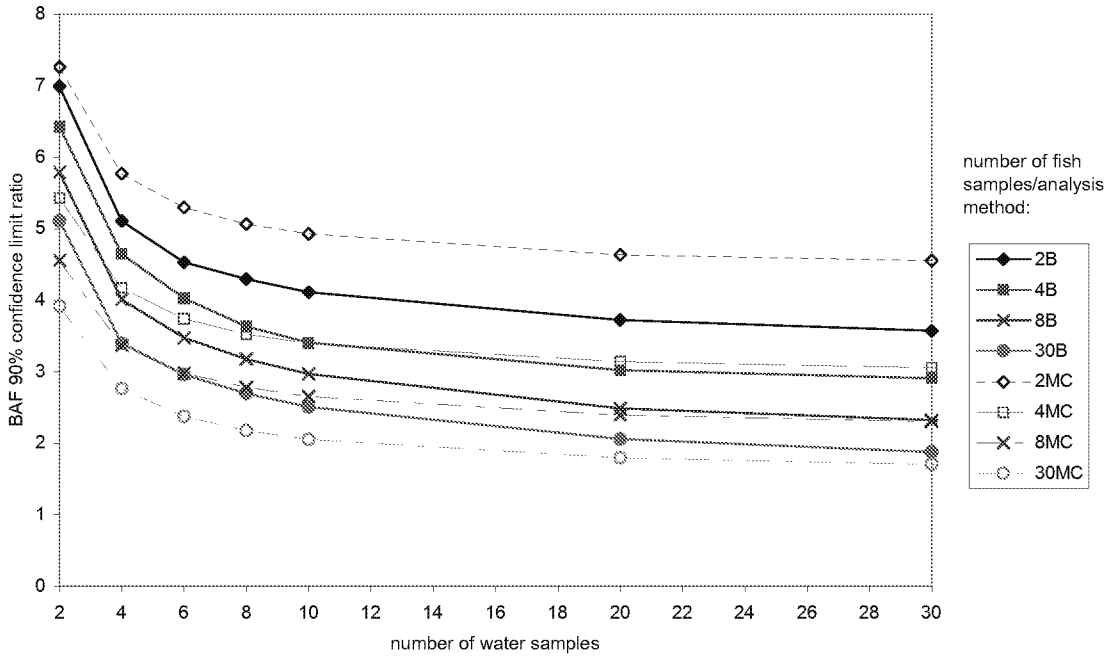

Figure 3C-1. Comparison of Monte Carlo and Bootstrap results: Ratio of 90% confidence limits for BAF as a function of numbers of fish and water samples for PCB congener 149 in Green Bay Zone 3 predator fish. Number of water samples are varied across x-axis; number of fish samples plotted as separate curves. Bootstrap resampling results are plotted as solid lines; Monte Carlo results as dashed lines.

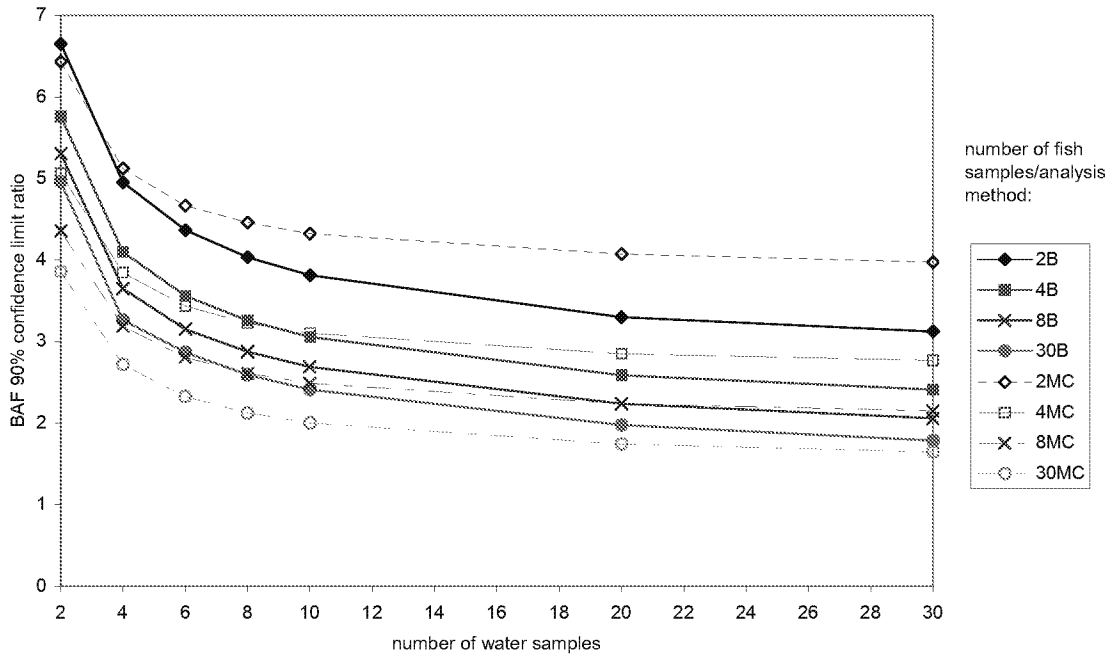

Figure 3C-2. Comparison of Monte Carlo and Bootstrap results: Ratio of 90% confidence limits for BAF as a function of numbers of fish and water samples for PCB congener 149 in Green Bay Zone 3 forage fish. Number of water samples are varied across x-axis; number of fish samples plotted as separate curves. Bootstrap resampling results are plotted as solid lines; Monte Carlo results as dashed lines.

What is the effect of correlation between fish and water concentrations on BAF estimates?

The Monte Carlo BAF analyses were repeated, using varying degrees of correlation between chemical concentrations in biota and water. Correlation coefficients ranged from 0.001 (no correlation) to 0.9 (high positive correlation). It is reasonable to expect the concentrations of bioaccumulative chemicals to be correlated in biota and water, although from the standpoint of statistical analysis it is more convenient to assume the concentrations are uncorrelated and independent.

Correlations between chemical concentrations in biota and water reduced the BAF confidence limit ratios, percent bias and RMSE for all congeners. The benefits increased with the

magnitude of the correlation, and were more beneficial for smaller sample sizes. This is illustrated in Figure 3C-3, which plots the predator fish BAF confidence limit ratios for congener 149 as a function of the concentration correlation coefficient for sample sizes of 2/2, 10/10 and 30/30 (n_b / n_w). These results show that assuming chemical concentrations in biota and water to be uncorrelated (regardless of whether they actually are) is a conservative approach to selecting numbers of samples for the purpose of determining site-specific BAFs.

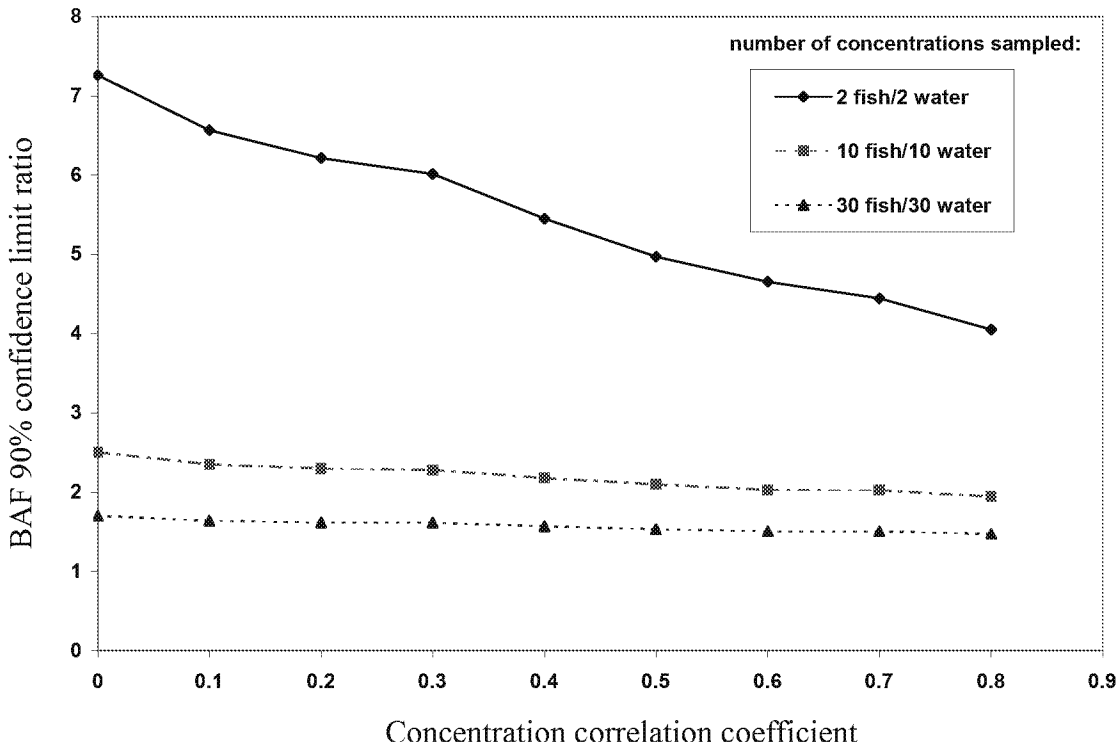

Figure 3C-3. Monte Carlo results for PCB congener 149 in Green Bay Zone 3 predator fish: Ratio of 90% confidence limits for BAF as a function of correlation between biota and water concentrations. The 3 curves are for simulations using different numbers of fish and water samples.

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How do Monte Carlo BAF results change if different variances for chemical concentrations in fish and water are assumed?

The Monte Carlo method described above was repeated using different degrees of variability in chemical concentrations in both fish and water. This was done to provide the investigator with general guidance for selecting sample sizes that would be applicable for different sites. Specifically, lognormal chemical concentration distributions, having coefficients of variation[10] (CV) ranging from 0.4 to 0.8, were specified independently for fish and water. For each case, the Monte Carlo method was used to calculate the 90% confidence limit ratio in BAFs. Selected results from this analysis are presented in Tables 3C-1 through 5. Each sub-table presents the 90% BAF confidence limit ratios as a function of the coefficient of variation (CV) of chemical concentration measurements in fish and water, for a specific number of fish and water concentrations. For example, Table 3C-1 is a tabulation of results for 2 fish and 2 water samples. For this case, highly-variable chemical concentrations result in 90% BAF confidence limit ratios that exceed 10. For moderately variable chemical concentrations, the BAF confidence limit ratios are mostly 5 or less.

Table 3C-1. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 2 Fish and 2 Water Samples

Fish concentration CV	Water concentration coefficient of variation (CV)				
	0.4	**0.50**	**0.60**	**0.70**	**0.80**
0.4	3.54	4.10	4.90	5.62	6.66
0.5	4.21	4.74	5.56	6.33	7.41
0.6	4.86	5.41	6.40	7.26	8.34
0.7	5.74	6.38	7.37	8.34	9.38
0.8	6.68	7.35	8.51	9.40	10.67

Table 3C-2 is a tabulation of results for 4 fish and 4 water samples. For this case, highly-variable chemical concentrations result in 90% BAF confidence limit ratios that exceed 5. For moderately variable chemical concentrations, the BAF confidence limit ratios are in the range of 3 to 4. For low-variability chemical concentrations, the BAF confidence limit ratios are mostly

[10] The coefficient of variation is the ratio of the standard deviation to the mean.

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smaller than 3. Results for 6 fish and 6 water samples are presented in Table 3C-3. In this case, highly-variable chemical concentrations result in 90% BAF confidence limit ratios that are in the range of 3 to 4, while BAF confidence limit ratios are less than 3 for low to moderately variable chemical concentrations. Once the number of samples exceeds about 6, the reductions in BAF confidence limit ratios become incrementally much smaller. Depending upon the requirements for BAF accuracy, exceeding sample sizes of 10 appears to be warranted only for sites having very high variability in chemical concentrations in fish and/or water.

Table 3C-2. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 4 Fish and 4 Water Samples

Fish Concentration CV	Water concentration coefficient of variation (CV)				
	0.4	**0.50**	**0.60**	**0.70**	**0.80**
0.4	2.47	2.77	3.07	3.49	3.89
0.5	2.74	3.06	3.41	3.80	4.23
0.6	3.11	3.39	3.75	4.20	4.65
0.7	3.49	3.79	4.18	4.59	5.02
0.8	3.92	4.33	4.64	5.12	5.65

Table 3C-3. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 6 Fish and 6 Water Samples

Fish Concentration CV	Water concentration coefficient of variation (CV)				
	0.4	**0.50**	**0.60**	**0.70**	**0.80**
0.4	2.08	2.29	2.54	2.80	3.08
0.5	2.29	2.49	2.71	3.00	3.25
0.6	2.50	2.73	2.96	3.21	3.55
0.7	2.78	3.00	3.21	3.52	3.81
0.8	3.11	3.33	3.56	3.82	4.10

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The tabulations for unbalanced sample numbers (i.e., n_b n_w) also demonstrate that it is most effective for the investigator to apply more sampling effort to the chemical concentration (biota vs. water) that is more variable. For example, compare Table 3C-4 (2 fish, 4 water concentrations) and 3C-5 (4 fish, 2 water concentrations). In the former case, highly-variable water concentrations result in lower BAF confidence limit ratios than do highly-variable fish concentrations. In the latter case the situation is reversed. In other words, additional sampling counteracts the tendency for highly-variable fish or water concentrations to inflate the BAF confidence interval ratio.

Table 3C-4. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 2 Fish and 4 Water Samples

Fish Concentration CV	Water concentration coefficient of variation (CV)				
	0.4	**0.50**	**0.60**	**0.70**	**0.80**
0.4	3.01	3.32	3.64	4.09	4.53
0.5	3.57	3.87	4.22	4.71	5.16
0.6	4.32	4.60	4.99	5.40	5.94
0.7	5.05	5.49	5.79	6.30	6.95
0.8	6.06	6.31	6.87	7.36	7.88

Table 3C-5. 90% Confidence Limit Ratios (Upper Confidence Limit/Lower Confidence Limit) for BAF as Functions of the Variability in Chemical Concentrations in Fish and Water: Chemical Concentrations Measured in 4 Fish and 2 Water Samples

Fish Concentration CV	Water concentration coefficient of variation (CV)				
	0.4	**0.50**	**0.60**	**0.70**	**0.80**
0.4	2.99	3.58	4.32	5.11	6.16
0.5	3.31	3.93	4.61	5.49	6.44
0.6	3.67	4.25	4.97	5.80	6.66
0.7	4.04	4.68	5.45	6.35	7.33
0.8	4.48	5.20	5.94	6.82	7.80

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Appendix 3D

Modeling Simulation of BAF Sampling Designs

Burkhard (2003) performed model simulations to understand how the variabilities in water and sediment chemical concentrations translate into the variabilities associated with BAFs (and BSAFs, which will be discussed separately in Section 4) based upon different sampling designs. Different models were constructed to evaluate temporal and spatial variability in chemical concentrations. As noted by Burkhard (2003), for these simulations to be meaningful the model constructs should provide reasonable representations of ecosystem conditions and chemical properties. Because the models are generic (i.e., not calibrated to site-specific data), the results are intended to compare different sampling designs in terms of the resulting BAF precision, and do not offer definitive predictions with known certainty.

Model for evaluating temporal variability

A model river segment with a point source discharge was constructed, and the total chemical load was assumed to be released from the point discharge. Daily instream chemical concentrations were calculated by using a simple dilution model based on the daily stream flow, the discharge flow, and the chemical concentration in the discharge. The model river segment was assumed to contain a food web consisting of zooplankton, benthic invertebrates, forage fish, and piscivorous fish. Three food web structures were considered: pelagic, where forage fish eat only zooplankton; benthic, where forage fish eat only benthic invertebrates; and mixed, where the diet of forage fish consists of 50% zooplankton and 50% benthic invertebrates. Time-variable chemical concentrations in forage and piscivorous fish were modeled by integrating the differential equations for the chemical mass balance within each organism (Gobas, 1993). Average chemical concentrations in the forage and piscivorous fishes were calculated each day, using the exposure concentrations from the dilution model. Chemical concentrations in the lower food web organisms (zooplankton and benthic invertebrates) were calculated using the modeling assumptions of Gobas (1993), which were equilibrium conditions with their respective environments (i.e., water column and sediment, respectively). The lower trophic levels serve as

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important "entry points" for hydrophobic organic chemicals into the aquatic food web. Details of the modeling approach can be found in Burkhard (2003). Although simplistic, this model's predictions of chemical concentrations in fish and how they vary in response to temporal variation in exposure concentrations is consistent with more contemporary and/or site-specific calibrated models.

Model for evaluating spatial variability

To evaluate the effect of spatial as well as temporal variability, the above river segment was divided into six subsegments with one subsegment receiving the point source discharge. Daily chemical concentrations in that subsegment were determined by using the method described previously for the model for evaluating temporal variability. Daily chemical concentrations in the other subsegments were determined as ratios of the concentration predicted in the modeled subsegment. Sediment chemical concentrations were set and held constant for the entire simulation by using the average water column chemical concentration in each subsegment (for the entire flow data set), and $_{socw}/K_{ow} = 1$.

Simulations were performed by randomly moving 100 piscivorous fishes up and down the river through the river subsegments. In sampling a specific subsegment, the chemical concentration in fish was obtained by averaging the chemical concentrations in all piscivorous fishes present in the subsegment at the sampling event. When calculating BAFs and BSAFs for sampling designs with multiple water, fish, and sediment samples, average chemical concentrations were used for each phase in the above equations.

Modeling results

Examples of the predictions made by the temporal variability model are shown in Figure 3D-1. Daily chemical concentrations in the river segment were calculated by using Mississippi River flow data for the 1995 calendar year (Figure 3D-1.a). A substantial change in chemical concentrations is observed around day 75, due to a rapid increase in daily flow rates, which were sustained through the first half of 1995. Chemical concentrations in piscivorous fish were calculated from the daily chemical concentrations in the river for chemicals with log K_{ow}s ranging from 2 to 9 (Figure 3D-1b). Comparison of the chemical concentrations in the river to

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the predicted concentrations in piscivorous fish reveals that concentrations in the fish will change (relative to the chemical concentrations in the ambient water) at rates that are dependent upon the hydrophobicity of the chemical. In all time-variable bioaccumulation models, the rate of change in chemical concentrations in biota is controlled by the overall rate of chemical loss from the organism. This rate is the sum of elimination rates via gills and gut, the organism growth rate, and the rate of chemical metabolism or biotransformation. The rate of change decreases with increasing K_{ow} because the elimination rates are modeled as inverse functions of hydrophobicity. For chemicals with low K_{ow}s (log K_{ow} <3), the rate of change is so fast that chemical concentrations in the fish will mimic the trends of the chemical concentrations in water, for example, compare the scaled concentrations for log K_{ow}s of 2 and 3 (Figure 3D-1b) to the daily chemical concentrations (Figure 3D-1.a). For chemicals with large K_{ow}s, chemical concentrations in the fish change slowly relative to changes in the chemical concentrations in the ambient water and will follow the long-term trends for the chemical concentrations in the river. For highly-hydrophobic chemicals, the organism growth rate becomes an important factor in determining the overall rate of chemical loss. From a field study design perspective, the rate at which the chemical concentrations in the fish change relative to the rate of change of the chemical concentration in river will strongly influence the design for the field study, for example, number of samples collected over what time period.

With the simulated daily chemical concentrations in fish and water and the chemical concentration in the sediment, hypothetical field-sampling designs were evaluated by sampling the simulated data as if one were actually performing a field study. Consider the simplest field design possible, the collection of fish, water, and sediment on one day. With the 1995 data, this field design can be performed 365 times, once for each day of the year. Computationally, when performed for all possible dates, this field design results in 365 BAFs. Consider another field design consisting of two sample collections spaced two weeks apart; this field-sampling design can be performed successfully 351 times with the 1995 data. When performed for all possible dates, this field design results in 351 BAFs. The distribution of these BAF values provides an estimate of the uncertainty of the BAF "measured" in the field sampling design being simulated.

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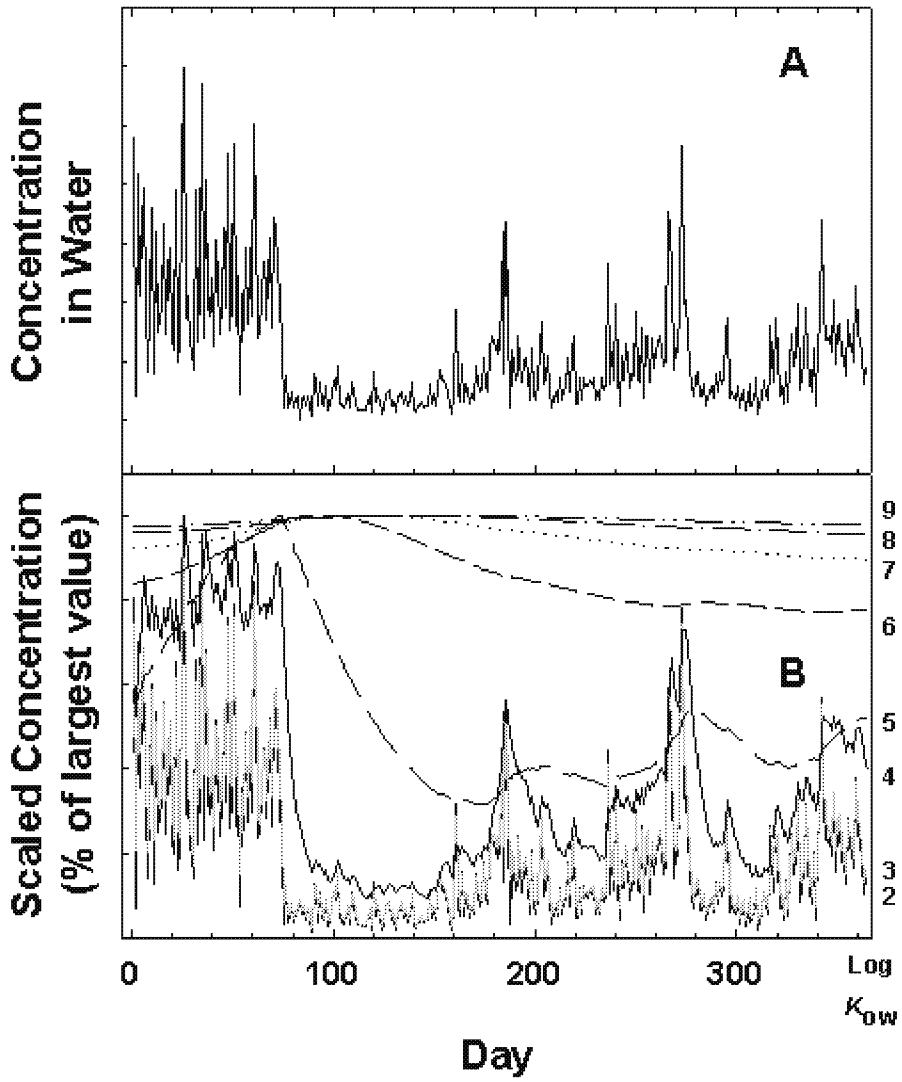

Figure 3D-1. (A) Daily chemical concentrations in the model river segment. (B) Daily chemical concentrations in piscivorous fish for chemicals with log K_{ow} s of 2, 3, 4, . . . , and 9. The daily chemical concentrations in piscivorous fish have been scaled to the largest value for each K_{ow}. The daily chemical concentrations for log K_{ow} s of 2 and 3, after scaling, are practically identical; the log K_{ow} data are plotted in gray.

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The ratio of the 90th to 10th percentile values of the distribution of BAF predictions was found to be a useful measure of variability. This ratio defines the range or width of the data, and smaller ratios result in smaller uncertainties for a given sampling design. For the example presented above, ratios of the 90th to 10th percentile BAFs of 8.63 and 5.94 were obtained for the daily and two-week sampling designs, respectively. These ratios easily demonstrate which design provides the lower uncertainties, on average, in the measured BAF. To summarize the overall process for evaluating field sampling designs, the following steps are performed: (1) determine the daily concentrations of chemical in the river; (2) compute the daily chemical concentration in the fish; (3) with the field designs of interest, sample the data for all possible dates, and calculate BAFs for all possible dates; (4) determine the ratio of the 90th to 10th percentile values; and (5) compare the ratios of the 90th to 10th percentile values to determine which designs resulted in the smallest uncertainty. Steps 3 and 4 were performed for a number of field designs.

Temporal variability in chemical concentrations

Sampling designs consisting of 1 to 14 sampling events to collect grab water samples with uniform spacing between water sample collection dates ranging from 1 to 60 days were evaluated by using the model river segment with Mississippi River flow data for the years 1955 through 1995, $_{socw}/K_{ow}$ =1, and the mixed benthic– pelagic food web. The results, in terms of the ratios of the 90th to 10th percentile BAF values, are plotted in Figure 3D-2. Two different sampling designs were considered for the collection of piscivorous fish. In the first series, piscivorous fish were collected once and their collection coincided with the date of the last collected water sample. This design is commonly used in many field studies because of the logistics of assembling a field crew for sampling fish and sediment. The second series consisted of the collection of piscivorous fish concurrently with each water sample.

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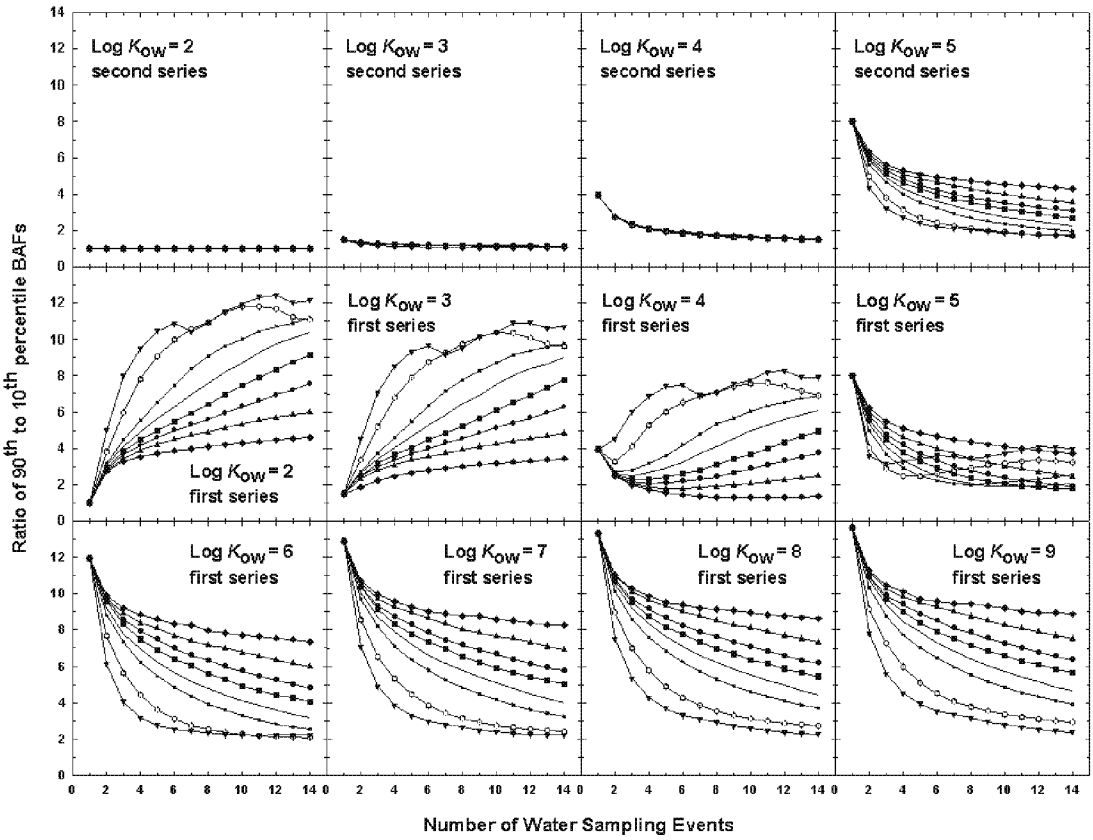

Figure 3D-2. Ratio of the 90th to 10th percentile bioaccumulation factors (BAFs) for field-sampling designs consisting of daily water samples spaced 1 ( ), 3 ( ), 5 ( ), 7 ( ), 10 (line), 14 (•), 30 (), and 60 ( ) d apart, with fish samples collected concurrently with the last water sample (first series) and with fish samples collected with each water sample (second series), when using Mississippi River (USA) flow data for years 1955 to 1995.

For chemicals with log K_{ow} s greater than 5, the ratios of 90th to 10th percentile BAFs for the first and second series were practically identical; therefore, only the results for the first series are reported in Figure 3D-2. For chemicals with log K_{ow} s greater than 5, increasing the number of water samples as well as the spacing between water sample collection dates reduces the uncertainty of the measured BAF. In contrast, for chemicals with log K_{ow} s of 4 or less, the second sampling design had smaller uncertainties.

If the above sampling designs are modified by the collection of composite water samples over time rather than using grab water samples, the most dramatic effect is observed when

limited numbers of samples are used; for example, four and fewer. For these designs, chemicals with log K_{ow}s of 5 and greater have smaller uncertainties in the measured BAFs in comparison to those determined using the grab water samples. However, as the number of water samples collected increases, the uncertainties associated with the grab water sampling designs approach those of the composite sample designs. For chemicals with higher K_{ow}s, only 1 or 2 additional samples are necessary to obtain the same uncertainty in BAFs as would be obtained by composite sampling of water. In other words, a sampling design using 7 or 8 water grab samples results in about the same BAF uncertainty as a sampling design using 6 water composite samples. For log K_{ow}s of 2 and 3, exactly the opposite behavior was observed between the grab and composite water sampling designs: compositing caused larger uncertainties in the measured BAFs for both series of sampling designs. For log K_{ow} of 4, the compositing sample designs provided lower uncertainties for the second design series, whereas rather mixed results were observed for the first design series. These results suggest that compositing sample designs are most useful for chemicals with log $K_{ow} > 4$.

The chemical concentrations used in the temporal variability simulations had a coefficient of variation of 118%. Burkhard (2003) found qualitatively similar results to those presented above were obtained when lower temporal variabilities were used. There may also be situations where the temporal variability in chemical concentrations is higher than 118%, and in this case the design of an appropriate field sampling plan will be more challenging. Such situations may include ecosystems subject to frequent or periodic storm events or tidal action, or systems with unusual sediment transport dynamics. As the temporal variability in chemical concentrations increases, so will the required number of water samples in order to accurately measure the average concentration. In ecosystems where high temporal variability in chemical concentrations is expected, the investigator should consider whether another method of determining the site-specific BAF, such as measuring a BSAF (method 2), is more appropriate in terms of accuracy and cost than measuring the BAF directly.

It is interesting to compare the modeling-based results of BAF uncertainty shown in Figure 3D-2 to the bootstrap resampling results from Section 3.2.1.3. For example, the ratio of

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90% exceedance limits estimated by bootstrap for PCB congener 149 (Figure 3B-4) can be compared to the modeling results for log
K_{ow} = 7 (the 10th panel in Figure 3D-2). Even though the exceedance limits are not the same (90% for bootstrap BAFs vs. 80% for modeled BAFs), the BAF ratios are quite comparable. For 2 widely-spaced water samples and a chemical in the range of $6 < \log K_{ow} < 7$, the simulated BAF confidence interval ratio is 6 to 7 versus the Bootstrap resampling ratio of 6.7 for PCB 149 ($\log K_{ow} = 6.67$). In addition, both methods produce the same trends in BAF ratio as a function of sample size. This is somewhat remarkable given that the two methods arrive at estimates of BAF uncertainty via completely different approaches, procedures, information and assumptions. The consistency of results lends credibility to both approaches.

Metabolism

When metabolism of a chemical occurs, measured BAFs will be smaller than those measured in the absence of metabolism (with the same hydrophobicity) because of the increase in the overall elimination or transformation rate of the chemical. Arnot and Gobas (2003) suggest that chemicals with metabolic biotransformation rates greater than 0.1 to 0.2 /day in fish do not appear to biomagnify in aquatic food webs. Examination of the results of the simulations suggests that when metabolism does occur, the appropriate sample design for a chemical of a given K_{ow} would be best described by the sample design for a chemical with a smaller K_{ow} (with no metabolism) and the degree of smallness is dependent upon the metabolism rate. In situations where metabolism rates cannot be reliably determined, the use of the second series of sampling designs would provide lower uncertainties for BAF measurements.

Food web structure and sediment–water chemical concentration relationship

The magnitude of a BAF is dependent upon a number of ecosystem and environmental parameters and conditions, notably food web length, food web composition, and the sediment-water column chemical concentration relationship. However, it is common to assume that these parameters and conditions are essentially fixed in a given ecosystem, in which case their influences upon the variability observed in a measured BAF are expected to be small. If any of these factors vary, the extent of bioaccumulation may change. However, we also expect that water concentrations usually change more rapidly than these other factors. Burkhard (2003)

ED_006490_00009970-00156

conducted sensitivity analyses which demonstrated that food web structure and sediment-water chemical concentration relationships are usually not important considerations that need to be factored into a sampling design.

Spatial variability in chemical concentrations

In the results discussed above, no spatial variability in chemical concentrations was considered. In most ecosystems, however, concentrations of chemical contaminants in sediments and water do vary spatially. To provide some insight into the importance of spatial variability, a series of random walk[11] simulations was performed with the model river segment, which was now divided into six subsegments. In these simulations, chemical concentration gradients spanned up to two orders of magnitude (Burkhard, 2003). Examination of the results (not shown) suggests that chemical concentration gradients do not add large uncertainties into the measured BAFs, beyond those caused by temporal variability alone.

Additional random walk simulations were performed to further evaluate the effects of spatial variability. These results suggested that BAFs can be measured with low uncertainty even when extreme spatial concentration gradients exist at the field site. However, these simulations also suggest that measurements for BAFs probably should be designed around the more contaminated reaches of the sites. When organisms are collected from the least contaminated sites, uncertainties of the BAF measurements can become very large for chemicals with log K_{ow}s between 3 and 5.

[11] Random walk is the idea of taking successive steps, each in a random direction. Treating motion (in this case, the movement of fish) as a random walk is a simulation tool used to simplify the complex ways objects (e.g., fish) move in nature.

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4. MEASURING BIOTA-SEDIMENT ACCUMULATION FACTORS TO PREDICT SITE-SPECIFIC BIOACCUMULATION FACTORS

Biota-sediment accumulation factors (BSAFs) are calculated from the concentrations of a chemical in tissue and sediment samples from the site of interest. BSAFs are expressions of net bioaccumulation by an organism as a result of uptake from all environmental sources and processes. A BSAF is similar to a field-measured BAF in that the chemical concentration in the biota sample reflects the organism's exposure through all routes. Also like a field-measured BAF, a BSAF accounts for bioavailability, because the sediment concentrations are normalized to the organic carbon content. Similarly, both field-measured BAFs and BSAFs account for chemical metabolism in the aquatic organism or its food web. Because of these similarities, a site-specific baseline BAF can be predicted from a BSAF, provided there is data on the distribution of the chemical between sediment and water at the site. This approach is Method 2 of EPA's national bioaccumulation methodology.

The purpose of Method 2 is to convert the bioaccumulation information contained in a measured BSAF to the corresponding baseline BAF value for a chemical. Prediction of a site-specific baseline BAF from a BSAF requires data for concentrations for multiple chemicals measured in ambient water and sediment from the site, preferably from a common sediment-water-biota data set. This method is useful when the concentration of the chemical of interest cannot be measured in the ambient water, and in some circumstances when it can. Method 2 is appropriate for moderate to highly hydrophobic nonionic organic chemicals, and certain ionic organic chemicals that exhibit lipid and organic carbon partitioning behavior similar to that of nonionic organic chemicals. Since this method specifically applies to organic chemicals, we will refer to site-specific baseline BAFs as “site-specific BAFs” in this section, although the reader should recognize that we are referring to baseline values throughout.

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4.1 DESCRIPTION OF METHOD 2

There are Three Steps Involved in Applying Method 2 to Determine a Site-Specific Bioaccumulation Factor:

1. Calculate a BSAF from the average concentrations of the chemical of interest in tissue and sediment samples from the site
2. Determine the distribution of concentrations between sediment and water at the site for one or more reference chemicals
3. Predict the site-specific BAF using the Method 2 equation (Equation 4-2)

The BSAF is defined as the ratio of the lipid normalized concentration of a chemical in an organism sampled at trophic level *i* to the organic carbon normalized concentration of the chemical in surficial sediment (Ankley et al., 1992):

$$BSAF_i = \frac{C_t / f_l}{C_s / f_{soc}} = \frac{C_l}{C_{soc}} \qquad \text{Equation 4-1}$$

where:

$BSAF_i$ = Biota-sediment accumulation factor for aquatic organism at trophic level *i*,

C_t = Chemical concentration in the organism [M_{chem}/M_{tissue}],

f_l = Lipid fraction of the organism,

C_s = Chemical concentration in surficial sediment [$M_{chem}/M_{sediment}$], and

f_{soc} = Organic carbon fraction of the sediment.

In addition:

C_l = Lipid-normalized concentration of the chemical in whole fish [M_{chem}/M_{lipid}], and

C_{soc} = Organic carbon-normalized concentration of the chemical in the surface sediment [$M_{chemical}/M_{organic\ carbon}$]

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The BSAF has units of kg of organic carbon/kg of lipid. The use of lipid and organic carbon normalized concentrations makes the BSAF an approximate fugacity[1] ratio (Burkhard et al. 2003a).

BSAFs for fish and other organisms not in intimate contact with the sediments can only be determined using field data. Meaningful BSAFs, i.e., values which enable accurate prediction of chemical residues in fish, require that the sediment samples be reflective of the organism's recent exposure history. In general, BSAFs should be determined from spatially and temporally coordinated fish and surficial sediment samples under conditions in which recent loadings of the chemicals to ecosystem are relatively unchanged (Burkhard et al. 2003a). Average chemical concentrations are used in the calculation of the BSAF as they are for BAFs, since multiple samples should be collected to properly characterize chemical concentrations at a site. The appropriate averaging method (e.g., arithmetic or geometric mean) depends upon the distribution of the concentration data, and should be selected following data review.

Both BSAFs and baseline BAFs can provide good measures of the relative bioaccumulation potential of hydrophobic organic chemicals if based on accurate measurements of concentrations in appropriate samples of biota, sediment, and water. When calculated from a common organism-sediment-water sample set, chemical-specific differences in BSAFs or baseline BAFs reflect the net effect of biomagnification, metabolism, bioenergetics, and bioavailability factors on each chemical's bioaccumulation.

Method 2 predicts the site-specific BAF from the measured BSAF for the chemical of interest, using the sediment-water concentration quotient determined for one or more similarly-behaving reference chemicals. Specifically, this method uses sediment-water concentration quotients ($_{socw}$ s) for reference chemicals to estimate values of C_w^{fd} that cannot be measured for the chemical of interest. Each chemical's K_{ow} should also be known, because the ratio of $_{socw}$ to K_{ow} provides the basis for relating reference chemicals to the chemical of interest. The

[1] Fugacity expresses chemical concentrations as a partial pressure, which indicates the tendency of a chemical to "prefer" one phase (e.g., lipid, organic carbon, dissolved) over another (Mackay, 1979).

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following equation is used to predict the site-specific baseline bioaccumulation factor for the chemical of interest from a BSAF measured at the site:

$$\text{Site-Specific Baseline BAF}_i = \text{BSAF}_i \left(\frac{D_{k/r}\ \Pi_{socw,\,r}\ K_{ow,k}}{K_{ow,r}} \right) - \frac{1}{f_l} \qquad \text{Equation 4-2}$$

The subscripts *k* and *r* refer to the chemical of interest and a reference chemical, respectively. Also:

$_{socw}$ = Sediment-water concentration quotient [L^3/M]

$D_{k/r}$ = Ratio of the fugacity gradients (modeled as $_{socw}$ / K_{ow}) between sediment and water for chemical *k* in comparison to that of a reference chemical *r*

The sediment-water concentration quotient, determined for one or more reference chemicals as the ratio of measuring chemical concentrations in sediment and dissolved in water at the site, is a critical parameter in predicting the baseline BAF from a BSAF. It is calculated as:

$$\Pi_{socw,\,r} = \frac{C_{soc,r}}{C^{fd}_{w,r}} \qquad \text{Equation 4-3}$$

where:

$C_{soc,r}$ = concentration of a reference chemical in dry sediment, normalized to sediment organic carbon

$C^{fd}_{w,r}$ = concentration of the reference chemical that is freely dissolved in water

Again, average chemical concentrations should be used in the calculation of the sediment-water concentration quotient, and appropriate averaging methods should be selected following review of the concentration data.

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The reference chemicals should have a hydrophobicity and organic carbon partitioning behavior similar to the chemical of interest, and the dissolved concentration of the reference chemicals in water must be quantifiable at the site. Octanol-water partition coefficients (K_{ow}s) are used to adjust for any differences in hydrophobicity between the chemical of interest and the reference chemicals. In an ecosystem at equilibrium, fugacity theory predicts that the $_{socw}$ should equal K_{ow} . Therefore, $_{socw}$ / K_{ow} is called the fugacity gradient. In many cases, the fugacity gradients between sediments and water for both reference chemicals and the chemical of interest are arguably similar. In fact, this similarity provides a useful criterion for the selection of reference chemicals. In cases where site-specific evidence suggests or demonstrates that fugacity gradients are not the same, the explicit difference may be represented by the fugacity gradient ratio, $D_{k/r}$:

$$D_{k/r} = \frac{\Pi_{socw,\,k} / K_{ow,k}}{\Pi_{socw,\,r} / K_{ow,r}} \qquad \text{Equation 4-4}$$

$D_{k/r}$ is an additional parameter that can be used to improve the accuracy of the BAF prediction by Method 2, if the necessary data are available for the chemical of interest and the reference chemicals. In practice, $D_{k/r}$ is often assumed to be 1.0.

In some situations, it may be possible to estimate or predict $_{socw}$ for the chemical of interest directly. This alternative is discussed in Section 4.4. If a reasonably certain estimate of $_{socw}$ is available for the chemical, the baseline BAF can be predicted directly from the BSAF using this simplification of the Method 2 equation:

$$\text{Baseline BAF}_i = \text{BSAF}_i\ \Pi_{socw} - \frac{1}{f_l} \qquad \text{Equation 4-5}$$

This seems like an attractive alternative, because data for reference chemicals has been eliminated from the equation. However, without measurements of $_{socw}$ for reference chemicals, it may be difficult or impossible to reliably estimate $_{socw}$ for the chemical of interest at the site. EPA prefers equation 4-2 and the use of reference chemical data for $_{socw}$ as being a more robust

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method for predicting site-specific BAFs. Calculating a site-specific BAF using Method 2 is presented in the following example.

Prediction of a site-specific BAF from BSAFs determined by measurements at the site (Method 2)

This example illustrates the development of a site-specific trophic level 4 BAF using Method 2. This method involves predicting a site-specific baseline BAF using a BSAF measured at the site for the chemical of interest, as well as the sediment-water concentration quotient for one or more reference chemicals. In Section 4.6, it is suggested that multiple reference chemicals be used to predict a site-specific baseline BAF with Method 2, because this improves the accuracy of the result.

In this example, data from Lake Ontario are used to derive a baseline BAF from a BSAF for PCB congener 126, which cannot be readily detected in water (USEPA, 1995; Cook and Burkhard, 1998). To simplify this example, a site-specific BAF is derived for only one trophic level 4 organism; in this case, age 5-7 lake trout. A review of the dietary preferences of the larger sizes of lake trout that are commonly consumed confirms that these are trophic level 4 organisms. Previously, the PCB congeners 52, 105, and 118 have been used as the reference chemicals for calculating baseline BAFs for PCB 126 (USEPA, 1995; Cook and Burkhard, 1995). These three congeners were selected because (1) they have similar physicochemical properties and are all from a single chemical class, (2) they are well quantified in sediment and biota, and (3) available data indicate they have loading histories similar to PCB 126 and thus the fugacity gradients ($_{socw}$ /K_{ow}) should be similar. In this example, the detailed, step-by-step calculations for each component of the equation are shown only for reference PCB congener 118. In practice, the same steps are performed for all reference congeners, but for this example, only the final site-specific baseline BAFs are shown for PCBs 52 and 105.

chemical	Log K_{ow}	C_l	C_{soc}	$C_w^{filtered}$
PCB 126	6.9	12.3 ng/g-*l*	3.83 ng/g-SOC	
PCB 118	6.7		555 µg/g-SOC	34 pg/L

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Prediction of a site-specific BAF from BSAFs determined by measurements at the site (Method 2, continued)

Determining a BSAF for the Chemical of Interest from Measurements at the Site

The BSAF for PCB 126 is determined as the ratio between the lipid-normalized concentrations of the chemical in 5 to 7-year-old lake trout (C_l) and the average organic carbon-normalized concentration of the chemical in surface sediment (C_{soc}) using equation 4-1. On the basis of data collected from Lake Ontario, the average C_l of PCB 126 in age 5-7 lake trout is 12.3 ng/g-lipid, and the average C_{soc} of PCB 126 in the sediment is 3.83 ng/g-organic carbon (actual calculations for these normalized values are not shown here). Therefore:

$$\mathrm{BSAF}_{4,\,PCB126} = \frac{C_l}{C_{soc}} = \frac{12.3ng}{g-lipid} \cdot \frac{g-oc}{3.83ng} = 3.21\ \frac{g-oc}{g\text{-}lipid} \qquad \text{Equation 4-1}$$

The trophic level 4 BSAF for PCB 126 in 5 to 7-year-old lake trout is 3.21.

Determining a Sediment-Water Concentration Quotient ($_{socw}$) for a Reference Chemical

Sediment-water concentration quotients for the reference chemicals can be determined from site-specific measurements by using equation 4-3. This calculation will be shown for one of the three reference chemicals, PCB 118. To calculate $_{socw}$, the concentration of reference chemical that is freely dissolved in water (C_w^{fd}) is needed. This concentration can be calculated from the freely dissolved chemical fraction (f_{fd}) (using equation 3-6) and the chemical concentration measured in the water column. The measured DOC concentration is 2.0 mg/L and the K_{ow} for PCB 118 = 5.01×10^6 (log K_{ow} = 6.7). Using equation 3-6, the freely dissolved fraction of PCB 118 in Lake Ontario water is calculated as follows:

$$f_{fd} = 1 / (1 + POC \cdot K_{ow} + 0.08 \cdot DOC \cdot K_{ow}) \qquad \text{Equation 3-6}$$

In this example, the chemical concentration was measured in a filtered sample, so POC is set equal to zero (assuming all particulates were removed by filtration):

$$f_{fd} = \frac{1}{1 + 0.08 \cdot \frac{2.0mg - DOC}{L} \cdot 5.01\times10^6 \frac{L}{kg} \cdot \frac{kg}{10^6 mg}} = 0.56$$

Prediction of a site-specific BAF from BSAFs determined by measurements at the site (Method 2, continued)

The concentration of PCB 118 measured in filtered Lake Ontario water is 34 pg/L. Thus, $(C_w^{fd})_{118}$ = 0.56×34 pg/L = 19 pg/L or 1.9×10^{-5} g/L.

The average $(C_{soc})_{118}$ = 555 g/g-SOC (sediment organic carbon). By substituting these values into equation 4-3, $_{socw}$ for the reference chemical (PCB 118) is calculated as:

$$\Pi_{socw,\,r} = \frac{C_{soc,r}}{C_{w,r}^{fd}} \qquad \text{Equation 4-3}$$

$$\Pi_{socw,118} = \frac{C_{soc,118}}{C_{w,\,118}^{fd}} = \frac{555\,\mu g}{kg-SOC}\cdot\frac{L}{1.9\times10^{-5}\,\mu g} = 2.9\times10^{7}\,L/kg-SOC$$

Calculating a Site-Specific Baseline BAF

A site-specific baseline BAF may be predicted from the field-measured BSAF for the chemical of interest (PCB 126) and $_{socw}/K_{ow}$ for each reference chemical using equation 4-2:

$$\text{Site-Specific Baseline BAF}_{4,126} = BSAF_{4,126}\,\frac{D_{126/r}\Pi_{socw,r}K_{ow,126}}{K_{ow,r}} - \frac{1}{f_l}$$

Since the loading histories and fugacity ratios of the chemical of interest (PCB 126) and the reference chemicals (PCBs 52, 105 and 118) are assumed to be similar, $D_{k/r} \sim 1$ in equation 4-2. To complete the calculation to predict the site-specific baseline BAF for PCB 126 using reference chemical PCB 118, the appropriate K_{ow} values for PCB 126 (7.9×10^6 or log K_{ow} = 6.9) and the fraction of lipid for lake trout (20% or 0.20) are entered into equation 4-2, along with the other terms which have been previously calculated:

$$\text{Site-Specific Baseline BAF}_{4,126} = 3.21\cdot\frac{(1)\left(2.9\times10^{7}\,\frac{L}{kg-SOC}\right)\left(7.9\times10^{6}\,\frac{kg}{L}\right)}{\left(5.01\times10^{6}\,\frac{kg}{L}\right)} - \frac{1}{0.20}$$

$$= 1.5\times10^{8}\ \text{L/Kg-lipid}$$

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Prediction of a site-specific BAF from BSAFs determined by measurements at the site (Method 2, continued)

The site-specific baseline BAFs using reference PCB congeners 52 and 105 are derived in the same manner as for PCB 118. The predicted site-specific baseline BAFs that result are 3.7×10^8 using congener 52 and 1.6×10^8 using congener 105. Once all the site-specific baseline BAFs have been predicted, the final site-specific baseline BAF is derived by calculating the geometric mean of the three baseline BAFs, which in this case is 2.1×10^8 L/kg.

Calculating a Site-specific Total BAF

In order to determine a water quality standard for PCB 126 in Lake Ontario, the site-specific baseline BAF must be converted to a site-specific total BAF. Recalling the relationship between the baseline BAF and the total BAF (BAF_t^T):

$$\text{Site Specific } BAF_{i,t}^{T} = (f_l \cdot \text{Baseline } BAF_i + 1) \cdot f_{fd} \qquad \text{(rearranged Equation 3-4)}$$

For PCB 126, the site-specific baseline BAF at trophic level 4 was calculated to be 2.1×10^8 L/kg-lipid. The freely dissolved fraction of PCB 126 in the Lake Ontario water column, which contains an average POC concentration of 0.075 mg/L, can be calculated using equation 3-6:

$$f_{fd,126} = \frac{1}{1+\frac{0.075\,mg-POC}{L}\cdot 7.94\times10^6\,\frac{L}{kg}\cdot\frac{kg}{10^6\,mg}+0.08\cdot\frac{2.0\,mg-DOC}{L}\cdot 7.94\times10^6\,\frac{L}{kg}\cdot\frac{kg}{10^6\,mg}} = 0.35$$

The average lipid content for lake trout was 20% or 0.20. With this information, the site-specific total BAF can be recalculated from the site-specific baseline BAF:

$$\text{Site-Specific } BAF_{4,T}^{t} = \left(0.20\cdot 2.1\times10^8\,\frac{L}{kg-l}+1\right)\cdot 0.35 = 1.4\times10^7\,L/kg$$

The site-specific total BAF for PCB 126 in Lake Ontario lake trout is 1.4×10^7 L/kg. This BAF for PCB 126 relates the *total* concentration of chemical in water to the *total* concentration of chemical in tissue of trophic level 4 organisms.

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Ecosystems are rarely at thermodynamic equilibrium, so a BSAF inherently includes a measure of the "disequilibrium" associated with the distribution of a chemical in the ecosystem. The deviation from the expected equilibrium value of approximately 1-2 (McFarland and Clarke, 1986) is determined by the net effect of all factors that contribute to the disequilibrium between sediment and aquatic organisms. A BSAF value greater than 1-2 can occur through biomagnification or when chemical concentrations in surface sediment have not reached steady state with those in water. A BSAF value of less than 1-2 can occur from diagenesis of organic carbon in sediments, kinetic limitations for chemical transfer from sediment to water or water to the food web, and biological processes (such as growth or metabolism/biotransformation of the chemical in biota or its food web). The influence of these ecosystem factors on the value of a BSAF is discussed in Section 4.6.2.

For high K_{ow} chemicals (e.g. log $K_{ow} > 6$), there are some distinct advantages of measuring BSAFs and using Method 2 to predict site-specific BAFs, over directly measuring site-specific BAFs. BSAFs are a more robust assessment tool for high K_{ow} chemicals, particularly when there is any meaningful benthic connection in the food chain. Another important advantage to emphasizing BSAFs when assessing bioaccumulation of high K_{ow} chemicals at a site is the ease and reliability of the measurements. Measurement of concentrations of most highly hydrophobic nonionic organic chemicals in sediment can be performed fairly easily. Consequently, with an appropriate BSAF, chemical residues in fish can be readily predicted using the concentration of the chemical of interest measured in sediment at the site. In contrast, measurements in water can be difficult due to temporal fluctuations of the chemical concentrations which are also often below method detection limits. Because concentrations of highly hydrophobic nonionic organic chemicals are temporally more stable in both fish and sediments, BSAFs better integrate fluctuating exposure conditions than do BAFs (Burkhard, 2003). For high K_{ow} chemicals, the relative concentration of chemical in the sediment is usually much larger than in the water. Hence, the analytical difficulties in accurately determining chemical concentrations in water are much greater than for sediment. In addition, the importance of chemical binding to particulate, colloidal, and dissolved organic carbon in water becomes much greater at high K_{ow}, making it more difficult to accurately determine the proportion that is freely dissolved.

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Method 2 appears to be particularly beneficial for predicting site-specific BAFs for chemicals such as polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), and certain polychlorinated biphenyl (PCB) congeners. These chemicals are often detectable in fish tissues and sediments but are difficult to measure in the water column and/or are subject to metabolism (biotransformation). Because BSAFs are based on field data and incorporate the effects of metabolism, biomagnification, growth, and other factors, site-specific BAFs estimated from BSAFs will reflect the net effect of all these factors.

Section 4 is organized in the following manner: Section 4.2 identifies key study design questions the investigator should address when planning a field study to measure BSAFs. Section 4.3 addresses how to determine $_{socw}$, either by measuring this concentration ratio for one or more reference chemicals, or by approximation or model prediction. Section 4.4 provides the investigator with two complimentary methods that can be used to design a sampling plan to measure BSAFs and $_{socw}$. Section 4.5 contains specific guidance for sediment sampling. Finally, a number of scientific issues associated with the use of BSAFs and the Method 2 prediction of BAFs are discussed in Section 4.6.

4.2 KEY STUDY DESIGN QUESTIONS FOR DETERMINING BIOTA-SEDIMENT ACCUMULATION FACTORS

The most important aspect of conducting a successful field study to measure BSAFs is collecting representative samples of the biota and sediment. The sediment samples should be representative of the surficial sediment within the home[2] range of the organism. Because of this, the home range of the target species will dictate the spatial scale of the sampling effort. Again, samples will be most representative when the measured concentrations of the chemical in biota and sediment are reflective of long term average concentrations. Temporal and spatial distributions of chemical concentrations, organism life history, and duration of exposure among

[2] As noted in Section 2.4, it may be more appropriate to sample sediment within the foraging range of the target organism.

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other factors all contribute to BSAF uncertainty and should be addressed by the field sampling plan.

Average chemical concentrations are used in the calculation of the BSAF, since multiple samples should be collected to properly characterize chemical concentrations at a site. Sampling requirements for biota and sediment will largely be controlled by the variability of chemical concentrations at the field site. As discussed in Section 3.3.4, chemical concentrations in biota vary both in space and time. In contrast, chemical concentrations in sediment generally exhibit significant spatial variability but little temporal variability, the latter often exhibited as a slow rate of change due to sediment erosion and deposition processes. Again, the properties of the chemical itself play an important role in defining this variability.

To predict a site-specific BAF from a BSAF determined at a site, the investigator should also accurately determine the other parameters required for Method 2 (Equation 4-2). These include the sediment-water concentration quotient for a reference chemical, the octanol-water partition coefficients for the chemical of interest and the reference chemical, and the fugacity gradient ratio. The sediment-water concentration quotient is discussed in the next section.

The investigator faced with designing a field study to determine a BSAF should consider the following series of key questions, intended to identify factors of the problem that should be addressed by the sampling plan. The application of data quality assurance procedures when measuring and applying BSAFs is very important.

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Key Factors to Consider When Designing a Field Study to Determine a BSAF and Predict a Site-Specific BAF

Characteristics of the Chemical of Interest

- Type of chemical – Method 2 is applicable to nonionic organic chemicals and similarly-behaving ionic organic chemicals
- Hydrophobicity
- Metabolism

What is an Appropriate Reference Chemical?

- Type of chemical: similar to chemical of interest
- Hydrophobicity: K_{ow} similar to chemical of interest
- Sensitivity of analytical methods (should be detectable in water and sediment)
- Should reflect steady-state conditions

What is an Appropriate Target Biota Species?

- Consumed by human population
- Size of consumed organisms
- Trophic level and prey items
- Relationship to sediment (i.e., benthic, epibenthic, pelagic)
- Lipid content
- Migration and movement (defines home range)

Characteristics of the Site

- Size of site / number of waterbodies
- Sampling characteristics (temporal and spatial variability of chemical concentrations)
- Ecosystem type
- Organic carbon (SOC, POC and DOC) concentrations
- Sediment deposition environment
- Spatial patterns of sediment type and chemical concentration distributions
- History and duration of chemical loading (chemical of interest and reference chemical)

Sediment Sampling

- Samples should be representative of target biota's recent chemical exposure (i.e., within home range)
- Sampling the surficial layer of sediment (upper 1-2 cm)

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Key Study Design Questions

Reference Chemicals. *Which reference chemicals are most appropriate to select for application of Method 2?*

- Similar bioaccumulation and partitioning behavior
- Similar hydrophobicity and fugacity (i.e., $_{socw}/K_{ow}$)
- Structural similarity
- Similar loading history and duration is preferred

Study Feasibility. *Can I adequately detect the chemical of interest in biota and sediment, and the reference chemicals in sediment and water, with available analytical methods (e.g., with a detection frequency > 80)?*

- Investigate detection limits of available analytical methods
- Compare to expected chemical concentrations

1. **Precision Goal.** *For an acceptable level of uncertainty in the site-specific BAF (e.g., within a factor of 10, a factor of 3,* ± 100 %, ...*), how do I determine the necessary level of effort (in terms of the number of samples to collect and analyze)?*

- DQO process (USEPA, 2000c)
- Monte Carlo simulations
- Bioaccumulation modeling

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4. Biota. *Which species should I sample?*

- Consider consumption patterns of the human population
- Availability of species at the site
- Diversity of exposure pathways (i.e., benthic & pelagic)
- Dietary composition/trophic status
- Ease of collection

5. Site Definition. *Have I adequately defined my site of interest in terms of spatial extent?*

- Spatial extent defined by home range of target species

The field data should be collected at the specific site for which the BSAF will be used to predict a site-specific BAF, and with the target species of concern. For large-scale sites, EPA recommends that biota and sediment samples be collected from each waterbody or ecosystem within the site for which BSAFs are to be derived.

6. Temporal Variability (i.e., Sampling Event Frequency). *How many times do I need to sample biota and sediment at the site?*

Biota Sampling Considerations

- Consider chemical properties (hydrophobicity and metabolism)
- Consider biota characteristics (migration, reproduction, availability, etc)
- Consider consumption pattern (e.g., times of year they are harvested)
- Lessons learned from bioaccumulation modeling

Sediment Sampling Considerations

- Normally sampled once
- Guidance on sampling events provided in discussion of Bioaccumulation Modeling (Section 4.4.1)

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7. **Spatial Variability (i.e., Number of Stations).** *How many locations should be sampled?*

- Consider evidence of spatial gradients in chemical concentrations due to pollutant sources and transport processes
- Biota characteristics (mobility/home range, habitat preference, etc.)
- Consumption characteristics (harvesting areas)
- Ecosystem properties (size of site, spatial differences in hydrodynamics, etc.)
- Consider spatial design options (e.g., random, stratified, systematic, judgment)

Once the number of sampling events is determined, the number of sampling stations/locations can be based on the number of samples required to obtain the desired precision (see Section 4.4.4).

8. **Biota Sample Type.** *What types of biota samples should I collect (i.e., age/size, tissue, quantity, etc)?*

- What ages/sizes of these species are consumed?
- Which tissues are most commonly consumed and how are they prepared?
- Does this vary with organism size?
- Composite vs. individual samples
- Chemical analysis requirements
- See Section 3.3 for discussion of biota sampling

9. **Sediment Sample Type.** *What types of sediment samples should I collect?*

- Define depth of surficial sediment to sample
- Individual grab samples vs. composites?
- Chemical analysis requirements
- See Section 4.6 for discussion of sediment sampling

10. **Chemical Analytical Methods.** *Which analytical methods should I use?*

- Must be specific for the individual chemical(s) of concern
- Must be able to measure and quantify ambient chemical concentrations (> 80% detection rate)

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11. **Biota Sampling Methods**. *How should biota be sampled?*

- Appropriate methods depend on waterbody and organisms

12. **Sediment Sampling Methods.** *How should sediment be sampled?*

- Appropriate methods depend on contaminant and waterbody
- Surficial samples vs. vertically-resolved cores
- Select proper sampling device

13. **Biota/Sediment Sampling Correspondence.** *How should I coordinate biota and sediment sampling (e.g., concurrent vs. staggered sampling)?*

- Consider chemical properties (hydrophobicity and metabolism)
- Consider ecosystem conditions (variability due to hydrodynamics) and temporal aspects of chemical loadings
- Lessons learned from bioaccumulation model simulations (Section 4.4.1)

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14. Ancillary Measurements. *What other parameters do I need to measure?*

- Lipid content in biota, organic carbon in sediment, and POC and DOC in water are required ancillary measurements
- Useful ancillary measurements for biota include age, sex, trophic status, stomach contents and tagging (stocking) information
- Total suspended solids (TSS) is a useful ancillary measurements for the water column
- Grain size, bulk density, and radioisotope concentrations (in core layers) are useful ancillary measurements for sediment
- Home/foraging range of target species

Method 2 also requires measurements of reference chemical concentrations in water. The investigator should refer to Section 3.4 for guidance on water sampling.

4.3 HOW CAN THE SEDIMENT/WATER COLUMN CHEMICAL CONCENTRATION QUOTIENT ($_{socw}$) BE DETERMINED?

In Section 4.1, the sediment-water concentration quotient ($_{socw}$) was introduced as a critical parameter in predicting the site-specific BAF from a BSAF using Method 2. The data that are generated as a result of a BSAF study reflect how the chemical of interest is distributed between biota, sediment and water by partitioning and fate mechanisms, in addition to bioaccumulation. In Method 2, the partitioning and fate factors are addressed by determining $_{socw}$, and using this parameter in the prediction of the site-specific BAF. In this Section, the investigator will find guidance regarding three methods to determine $_{socw}$: measuring concentrations of one or more reference chemicals in water and sediment at the site; estimating $_{socw}$ /K_{ow} as approximately the ratio of the fraction of organic carbon in water column particulates (f_{oc}) to that in surficial sediment (f_{soc}) by assuming steady state conditions; and predicting $_{socw}$ for a chemical of interest using a properly calibrated and confirmed fate and transport model.

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Alternatives for determining socw:

- Measuring site-specific chemical concentrations in water and sediment for one or more reference chemicals
- Estimating socw /K_{ow} as (f_{oc}/f_{soc}) by assuming steady state
- Predicting socw using transport and fate models

The distribution of a chemical between the sediment and overlying water at a site is described by the sediment-water concentration quotient (socw), which was defined in Equation 4-3. By expressing the concentration of chemical in sediment on an organic carbon normalized basis and the concentration of chemical in water on a freely dissolved basis, socw is a measure of the degree to which the chemical's distribution between the surface sediment and the water column approaches or deviates from a condition of thermodynamic equilibrium for the waterbody. The degree of disequilibrium (departure from equilibrium) is proportional to the degree to which the fugacity gradient (socw/K_{ow}) for the chemical diverges from a value of 1.0 (socw= K_{ow}). This assumes that (1) the organic carbon partition coefficient (K_{oc}) is the same in both the water column and the sediment and (2) K_{oc} is also equal to the K_{ow}. These assumptions and the empirical data upon which they are based, are discussed in TSD Volume 2, Sections 4.2.4 and 4.3.

In the aquatic environment, three factors are primarily responsible for causing socw to differ among ecosystems. First, the concentration distribution of nonionic organic chemicals in the water column and sediment are the result of well-known fate and transport processes, such as particle sedimentation and resuspension, chemical sorption to and desorption from suspended and bed sediments, volatization, biological/chemical transformation, and water column transport. These processes vary among ecosystems (O'Connor, 1988a-c). Second, the chemical loading history to the ecosystem plays an important role in its socw. For example, increasing the loading of a chemical to the water column causes an immediate rise in the concentration of the chemical in the water, and over time, the concentration of the chemical in the sediment will gradually increase through sedimentation processes. If the loading of a chemical to the water column is decreased, the concentration of the chemical in the water column drops quickly, whereas the

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concentration of the chemical in the sediments decreases slowly through burial of older and more contaminated sediments by newer and less contaminated sediments (Endicott and Cook, 1994; USEPA, 2003). Third, differences in organic carbon content in water column particulates (or suspended solids) and surface sediment vary among ecosystems (Gobas and MacLean, 2003; Burkhard, 2003a). The ratio of organic carbon contents (water column to surface sediment) approximates the steady-state value of $_{socw}/K_{ow}$ for the ecosystem due to diagenesis processes on the newly deposited surface sediments.

4.3.1 Measuring Site-Specific Reference Chemical Concentrations in Water and Sediment

The most direct and accurate way to determine $_{socw}$ is based on measurements for appropriate reference chemicals at the site. As described above, the factors that are primarily responsible for causing $_{socw}/K_{ow}$ to vary tend to affect related chemicals in similar ways. Reference chemicals with $_{socw}/K_{ow}$ similar to that of the chemical of interest are preferred for Method 2 and often are available. Theoretically, the difference between sediment-to-water fugacity ratios for two chemicals, "*k*" and "*r*" ($D_{k/r}$), can be used when reliable reference chemicals that meet the fugacity equivalence condition (i.e., $_{socw,k}/K_{ow,k} \approx {}_{socw,r}/K_{ow,r}$) are not available. Related nonionic organic chemicals, approximately at steady state, should have similar, if not equal, values of $_{socw}/K_{ow}$ that are related to the f_{poc}/f_{soc} ratio. When steady-state conditions are not present, as is often the case, $_{socw}/K_{ow}$ values for related chemicals may be similar. The similarity of $_{socw}/K_{ow}$ for two chemicals can be indicated by similarities in molecular structure, which imply similar physical-chemical behavior in water (e.g., hydrophobicity, persistence, and volatility), similar mass loading histories, and similar concentration profiles in sediment cores.

The investigator should consider the following factors when selecting reference chemicals for measuring $_{socw}$:

1. The reference chemicals and the chemical of interest should have similar physicochemical properties, as well as similar persistence in water and sediment. In addition, the reference chemicals and the chemical of interest should have similar

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chemical structures (i.e., the investigator should not select an alkane as a reference chemical for a polycyclic aromatic hydrocarbon).

2. Obtaining $_{socw,r}$ data for several reference chemicals with similar K_{ow}s (log K_{ow} ± 0.5) from the same water and sediment samples is preferable and will ensure that predictions are more robust than those that would be obtained with only one reference chemical.

3. Data for several reference chemicals and the chemical of interest should come from a common organism-water-sediment data set for a particular site. Preferably, $C_{soc,r}$ and $C_{soc,i}$ should be measured from the same sediment samples, because this eliminates uncertainty attributable to spatial heterogeneity of C_{soc}.

4. The K_{ow} value for the target and reference chemicals should be selected as described in Section 4.2.5 of TSD Volume 2 (USEPA, 2003).

5. Whenever possible, the loading histories for the reference chemicals and the chemical of interest should be similar, such that their sediment-water disequilibrium ratios ($_{socw}/K_{ow}$) would not be expected to be substantially different (i.e., $D_{k/r} \sim 1$). For example, a contaminant produced by combustion processes over hundreds of years (e.g., pyrene) should not be used as a reference chemical for a recently-introduced contaminant (e.g., brominated diphenyl ether).

6. Guidelines for sampling and measurement of $_{socw}$ are identical to those for sampling and measurement of C_w^{fd} under BAF Method 1, as described in Section 3.4, and C_{soc} under BAF Method 2, as described in Section 4.5. Because concentrations of bioaccumulative chemicals in surficial sediments are relatively constant on an annual basis in most carbonaceous, fine-sediment depositional areas, determination of an appropriate average C_w^{fd} in systems with temporal fluctuations is the greatest challenge in measuring $_{socw}$.

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7. POC and DOC should be measured in the same samples used to measure chemical concentrations in water, and organic carbon should be measured in the samples used to measure chemical concentrations in sediment.

Depending on the chemical of interest, it may be challenging to find suitable reference chemicals. In some cases, this may lead the investigator to use a different method to determine a site-specific BAF.

4.3.2 Estimating $_{socw}/K_{ow} \sim f_{oc}/f_{soc}$ by Assuming Steady State

Over the long term, the asymptotic (steady state) value for $_{socw} / K_{ow}$ can be approximated as the ratio of the fraction of organic carbon in water column particulates to that in surficial sediment (Burkhard et al., 2003a). As these authors point out, $_{socw} / K_{ow} = 1$ at equilibrium. However, in natural systems the sediments and water column are almost never at equilibrium and deviations of $_{socw} / K_{ow}$ from 1 can be used to describe this disequilibrium (or nonequilibrium) condition. When the disequilibrium is less than one (i.e., $_{socw} / K_{ow} < 1$) the chemical concentration in the water column is enriched relative to that in the sediment. When the disequilibrium is greater than one (i.e., $_{socw} / K_{ow} > 1$) the chemical concentration in the sediment is enriched relative to that in the water column.

Water column particulates (i.e., suspended solids) in most ecosystems have organic carbon contents that are higher than the organic carbon contents of their corresponding sediments. This is because, as particles settle and become incorporated into the sediments, the more labile portions of the organic carbon (e.g., carbohydrates and lipids) are converted to CO_2 by microbial and other processes associated with diagenesis. The loss of organic matter without concomitant chemical loss effectively increases C_{soc} in the sediment so that ecosystems tend to exceed equilibrium between surficial sediments and the water column; i.e., $_{socw} / K_{ow} > 1$ (Gobas and MacLean, 2003). Such disequilibrium between sediment and water creates a thermodynamic gradient for chemical to move back into the water column, but chemical exchange between sediments and overlying water is slow, so disequilibrium is maintained. The magnitude of this natural disequilibrium for lakes appears to increase with increasing water

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depth due to increased organic carbon mineralization (Gobas and McLean, 2003). For ecosystems at steady-state, this disequilibrium approximates the ratio of the greater organic carbon content of the suspended solids to the lesser organic carbon content of the sediments. Therefore, the expected steady-state for an ecosystem is not thermodynamic equilibrium ($_{socw}$ / K_{ow} = 1), but rather a $_{socw}$ / K_{ow} of approximately the ratio of organic carbon content in suspended and sediment particles.

The magnitude of the differences in sediment and water column particulate organic carbon contents in aquatic ecosystems is strongly influenced by the hydrodynamics of the ecosystem, because particle sedimentation, resuspension, and burial are directly controlled/influenced by the hydrodynamics of the ecosystem. Ecosystems with high resuspension rates (e.g., rivers) would most likely have smaller differences in organic carbon contents than ecosystems with lower resuspension rates (e.g., large lakes and reservoirs). Based upon typical organic carbon contents in aquatic environments (1–15% in water column particulate and 0.5–4% in sediment: Thurman, 1985; Ittekkot, 1988;Wong et al. 2001; Reschke et al. 2002), steady-state $_{socw}$ / K_{ow} values are expected to range from approximately 2 to 10. The lesser value of 2 arises due to minimum expected differences in organic carbon content of particulate matter in the water column and sediments. The greater value of 10 allows for effects of chemical gradients and greater relative organic carbon amounts in the water column.

4.3.3 Using Transport and Fate Models to Determine the Fugacity Gradient Ratio

The third major factor influencing sediment–water disequilibrium is loading history. The previous discussion was based upon steady-state conditions, but because the mass transfer rate of chemical between sediment and water column can be slow, steady-state conditions may not be achieved quickly. If chemical concentrations in sediment at the site are far from steady state due to recent changes in loading, then $_{socw}$ / K_{ow} cannot be approximated as ~ f_{oc}/f_{soc}. Furthermore, differences in loading history between the chemical of interest and available reference chemicals can also complicate the use of Method 2 to predict a site-specific BAF, because the investigator must then determine the appropriate value for the fugacity gradient ratio, $D_{k/r}$. In these cases, a transport and fate model can be very helpful in evaluating nonsteady-state conditions.

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Transport and fate models are tools that are useful for simulating chemical concentrations in water and sediment, and their rates of change in response to changes in chemical loading (Chapra, 1997; Thomann and Meuller, 1987). Consequently, these models can be used to simulate and predict $_{socw}$ in response to changing chemical loading. For example, Burkhard et al. (2003a) used a relatively simple transport and fate model to predict time-dependent chemical distributions between overlying water and surface sediments (i.e., $_{socw}$) in a lake as a function of chemical loading rates. The transport and fate model was a two-compartment dynamic mass balance model consisting of a completely mixed water column and an underlying surficial sediment layer. The model assumed complete mixing in the water column transport and accounted for inflow and outflow, solids settling, sediment resuspension and burial, diffusive exchange between the sediment and water column, chemical volatilization and photolysis, and time-variable chemical loading rates. Ecosystem parameters and conditions representative of Lake Ontario were taken from Endicott et al. (1990). This model and similar models (Thomann and DiToro, 1983; Mackay, 1989; Gobas et al. 1995; DePinto et al. 1998) have been calibrated and confirmed in the Lake Ontario ecosystem for a number of organic chemicals, including chlorinated pesticides, PCBs, PCDDs and PCDFs. Equilibrium partitioning of chemical in the sediment and water column between the particulate, dissolved organic carbon, and freely dissolved compartments was assumed, and particulate and dissolved organic carbon partition coefficients were estimated using the relationships described in the 2000 Human Health Methodology (USEPA, 2000). The model was used to predict chemical concentrations in sediment and the water column for a given time and loading rate.

The importance of chemical loading on $_{socw}$ is illustrated in Figure 4-1 for three different loading scenarios: (a) constant loading of a chemical to the ecosystem over time, (b) constant loading of chemical to the ecosystem with a doubling of loading at year 50, and (c) constant loading of chemical to the ecosystem with an 80% reduction in loading at year 50. These figures were based on predictions made for a nonmetabolizable chemical with a log K_{ow} of 6, using the model described above. In all three loading scenarios, the concentration of the chemical in the water column responds quickly to the change in loading, in contrast to the relatively slow response of the concentration of chemical in sediment. In these scenarios, sediment and water column particulates had organic carbon contents of 3% and 15%,

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respectively ($f_{oc}/f_{soc} = 5$). In all three scenarios, $_{socw}/ K_{ow}$ reaches a plateau at a value of 4.91, nearly equal to the f_{oc}/f_{soc} ratio.

Scenario (c) is applicable to many chemicals such as PCBs and DDTs which are no longer manufactured or used, but are often found to be present in sediments at concentrations that exceed thermodynamic equilibrium with the water column. The latter portion of scenario (c) illustrates how $_{socw}$ changes over time. Differences in ecosystem parameters and conditions, such as hydraulic retention rates, sedimentation and resuspension rates, water column and surficial sediment layer volumes, and chemical loading rates between ecosystems, affect the specific time scales and slopes of the changes in C_w^{fd}, C_{soc}, and $_{socw}$ associated with changes in chemical loading over time.

Transport and fate models have been applied in many waterbodies, including Great Lakes Areas of Concern (AOCs), Superfund sites, and impaired waterbodies identified under Section 303(d) of the Clean Water Act. These models may be based on widely-available computer programs such as WASP7 (Wool et al. 2001), EFDC (Hamrick, 1996), and AQUATOX (Park et al. 2004), or proprietary modeling programs. Considerable effort and expertise is required to develop credible and reliable models of chemical fate and transport. Such models must be properly calibrated and confirmed to site-specific data in order for the investigator to have confidence in the results. Guidance on model calibration and confirmation is currently under development by EPA's Council for Regulatory Modeling (http://cfpub.epa.gov/crem/).

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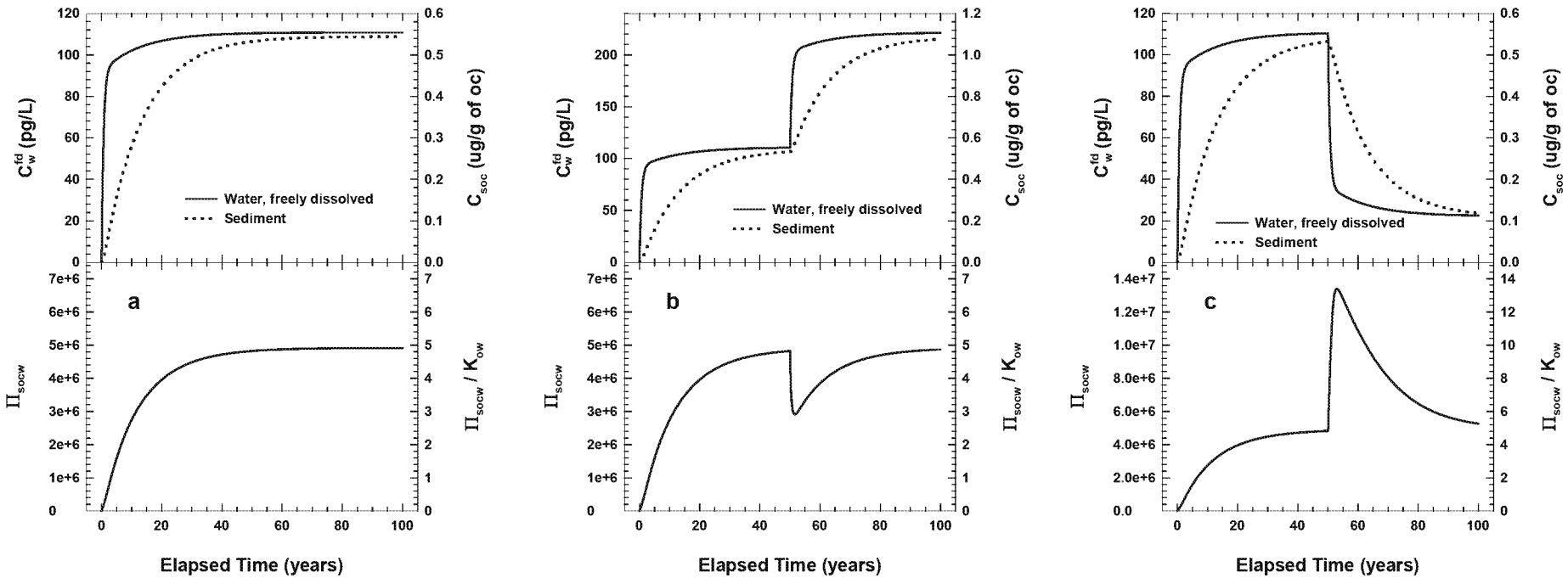

Figure 4-1. The sediment-water concentration quotient ($_{socw}$) for three different chemical loading scenarios: (a) constant loading of a chemical to the ecosystem overtime, (b) a constant loading of chemical to the ecosystem with a doubling of loading at year 50, and (c) a constant loading of chemical to the ecosystem with an 80% reduction in loading at year 50. Simulations performed for a chemical with a log K_{ow} of 6 using Lake Ontario conditions and parameters.

4.4 HOW TO DESIGN A SAMPLING PLAN TO MEASURE BSAFs

To design a field study to measure BSAFs at a site, the investigator should determine the appropriate number of biota, sediment, and water samples to collect as well as how to distribute them in time and space. The investigator should define the frequency of sample collection (i.e., the number and spacing of sampling events in time), and the spatial distribution of sample collection locations. Having the appropriate sampling frequency and spatial distribution (the sampling design structure) is critical for the determination of a BSAF that is representative of the long-term average conditions in an ecosystem. The collection and analysis of representative samples is the key to determining an accurate and unbiased BSAF, while the precision of a BSAF depends upon the number of samples. In the optimization process, the precision of the chemical concentration averages are balanced against the costs associated with sample collection and analysis. In many cases, compositing of samples is required to limit costs associated with the chemical analyses.

4.4.1 How Can Modeling Simulations Guide the Sampling Design Process?

The following approach to designing a field sampling program to measure BSAFs has been described by Burkhard (2003). First, the investigator should determine or reliably estimate the temporal variability of chemical concentrations at the site, as well as the chemical's K_{ow} and rate of metabolism. Second, an assessment of the immediate home range of the biota is required along with an assessment of spatial variability in chemical concentrations in sediment across this range. Third, the investigator should define the required precision for the BSAF measurement. With this information, the investigator can determine an appropriate sampling design structure (i.e., number of sampling events over time and space), for the chemical and site of interest. With the sampling structure delineated, the total numbers of samples can then be determined based upon the desired precision required for the BSAF measurement. Steps 1 through 4 of this approach are discussed in this section; step 5 is discussed in Section 4.4.4.

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BSAF Field Sampling Design Approach:

1. Determine the temporal variability of chemical concentrations at the site;
2. Assess the immediate home range of the biota and the spatial variability in chemical concentrations in sediment within this range;
3. Define the required precision for the BSAF measurement;
4. Select the appropriate sampling design structure; and
5. Calculate total numbers of biota and sediment samples, and allocate them among sampling events

The BSAF field sampling design approach is based on model simulations which demonstrate how variabilities in chemical concentrations translate into the uncertainties associated with BSAFs in different sampling designs. In the model simulations, fish, water, and sediment concentrations were predicted on a day-to-day basis with different ecosystem conditions and chemical properties. In these evaluations, model simulations were used to evaluate how temporal and spatial variability in chemical concentrations, the chemical's hydrophobicity, the chemical's metabolism rate in fish, the structure of the aquatic food web (benthic vs. pelagic components), and the disequilibrium between the sediment and water column for the chemical influence the number and timing of sampling events and their spatial distribution required to accurately and representatively determine BSAFs. The modeling approaches used by Burkhard (2003) to evaluate temporal and spatial variability in chemical concentrations were described in Section 3.2.2 and Appendix 3D, and the investigator is referred to that Section for details. In the simulations used to evaluate BSAF sampling designs, chemical concentrations in sediment were calculated as the product of the $_{socw}$ and the average chemical concentration in water for the time period (i.e., sampling interval) of interest. Presentation and discussion of the results, as they pertain to BSAF sampling designs, are presented in Appendix 4A.

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4.4.2 Using Model Simulations to Develop Field-Sampling Designs

Burkhard's (2003) simulations provide substantial insight into appropriate sampling design structures for BSAF measurements, which are generally *opposite* of those for measuring BAFs:

- For chemicals with log K_{ow} of 5 and less, with any rate of metabolism, appropriate BSAF sampling design structures would consist of numerous concurrent sets of sediment and fish samples spaced widely over time.

- For nonmetabolizable chemicals with log K_{ow} greater than 5, the collection of one concurrent set of sediment and fish samples would, in all likelihood, be an appropriate BSAF sampling design structure.

- With increasing chemical metabolism rate, appropriate BSAF sampling design structure transitions from the single concurrent collection of sediment and fish samples to designs appropriate for lower K_{ow} chemicals, that is, the collection of numerous concurrent sets of sediment and fish samples spaced widely over time.

Chemicals with intermediate metabolism rates (0.01 d^{-1} to 0.001 d^{-1} , corresponding to metabolic half-lives of 50 to 500 days) and/or moderate hydrophobicities ($4 < \log K_{ow}$ of < 6) present difficult challenges when selecting an appropriate sampling design structure for measuring BSAFs, as they do for BAFs. This range of hydrophobicities lies within the transition zone between the much more obvious design structures appropriate for low and high K_{ow} chemicals.

The process for developing successful field-sampling structures for BSAF measurements can primarily focus upon three parameters: temporal variability, metabolism, and K_{ow}, as was the case for measuring BAFs. These three parameters can range widely, and depending upon their values, dramatically different field designs would result. Although spatial variability is not usually a predominant factor in sampling design, knowing or understanding the immediate home range of the sampled organisms is required. Without this information, the investigator cannot ascertain whether sediment samples have been collected that are reflective of the actual chemical exposure history for the sampled organisms. Poor spatial coordination of fish and sediment

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samples will likely yield BSAFs with poor accuracy and large biases. In addition, the samples collected to measure BSAFs should be designed around the more contaminated regions within the site. Burkhard (2003) noted that BSAFs can be measured with low uncertainty even when spatial concentration gradients at the site are extreme, if these guidelines are followed carefully.

The sampling design structure (i.e., number of sampling events over time and space) can be developed for the chemical and ecosystem of interest based upon Burkhard's (2003) modeling simulations. Using the modeling results as a guide, some illustrative BSAF sampling structures have been developed (Table 4-1). These illustrative designs provide a sense of how sampling design structures might be influenced by differences in temporal concentration variabilities, metabolism rates, and K_{ow} s. The modeling results do not reflect the total uncertainty for the illustrative designs, because biases and errors in sampling, compositing, and chemical analysis were not included. Furthermore, temporal variabilities typical of other ecosystems (e.g., estuaries, reservoirs, lakes, and small streams) have not been evaluated. Additionally, the simulations were made using data for the entire calendar year, and field sampling is typically performed during warmer weather or better weather conditions. Although the illustrative sampling structures suggest the number and spacing of sampling events for a field study, they do not prescribe the total number of samples required for a successful BSAF field study.

The effects and importance of the immediate home range of the fish are also not included in the illustrative sampling structures (Table 4-1). Although spatial variability of the chemical in the ecosystem is not directly included in the illustrative sampling structures, sample collection for each sampling event should span the home range of the organisms in the ecosystem. Depending upon species, the home ranges are different; larger fishes tend to have larger home ranges (as discussed in Section 3.3.2). By collecting samples across the organism's home range, a truer picture of the average chemical exposures to the organisms of interest will be obtained. The ideal situation for determining a BSAF is when there are minimal concentration gradients across the organism's home range at the site. However, random walk (migration) simulations suggest that BSAFs can be measured with low uncertainty even when extreme spatial concentrations exist at the field site, provided the measurements are performed in more contaminated locations of the site.

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Table 4-1. Some Illustrative Biota–Sediment Accumulation Factor Sampling Design Structures. All Sampling Events are Assumed to be Widely Spaced in Time (e.g., 60, 90, 120 or 180 Days).

Metabolism rate	Temporal variability	log K_{ow}	Minimum number of sampling events[a]
low	high	$\leq 3, 4, 5, \geq 6$	8, 5, 2, 1
low	medium	$\leq 4, \geq 5$	2, 1
low	low	$\leq 3, \geq 4$	2, 1
medium	high	$\leq 5, 6, \geq 7$	8, 4, 1
medium	medium	$\leq 5, \geq 6$	4, 1
medium	low	$\leq 3, \geq 4$	2, 1
high	high	all K_{ow}s	9
high	medium	all K_{ow}s	4
high	low	all K_{ow}s	2

[a] The first value corresponds to the first value in the log K_{ow} column, the second value corresponds to the second value in the log K_{ow} column, etc.

4.4.3 Using Monte Carlo Simulation to Determine the Number of Samples to Collect and Analyze

In Section 3.3.1, the use of the Bootstrap and Monte Carlo simulations was demonstrated to estimate the number of biota and water samples required to determine a site-specific BAF of a known precision. The investigator can also use these methods to estimate how the precision of the BSAF depends upon the number of biota and sediment samples, and how the precision of the sediment-water concentration quotient ($_{socw}$) depends upon the number of sediment and water samples. Furthermore, if chemical concentrations in biota (for the chemical of interest and the reference chemical), sediment (chemical of interest) and water (reference chemical) are all simulated using Monte Carlo, the investigator can use the results to determine how the precision of site-specific BAF predictions made using Method 2 depend upon the number of samples collected from each medium at the site.

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Monte Carlo simulations of BSAF precision are demonstrated by examples in Appendix 4B, based again on Green Bay Mass Balance data for PCB congeners 18, 52, 149 and 180. Monte Carlo simulations of PCB congener concentrations were made using lognormal distribution moments (mean and CV) as measured in Green Bay zone 3 for dissolved water, lipid-normalized predator fish, and organic-carbon normalized surficial sediment.

The uncertainty of BSAFs and $_{socw}$ calculated in the Monte Carlo simulations were sensitive to the number of sediment samples, and this sensitivity increased with the variability of the sediment chemical concentrations. The variability of chemical concentrations in sediment affected the uncertainty of BSAFs and $_{socw}$, particularly for small sediment sample sizes (n_s 6). For highly variable chemical concentrations in sediment, increasing the number of sediment samples used to calculate the mean concentration had a significant impact on reducing the uncertainty of BSAFs, up to a sample size of about 6. Collecting additional sediment samples (i.e., greater than 10) had little effect on the precision of BSAFs.

For BAFs derived using Method 2, the results were similar to those for BSAF and $_{socw}$, although the confidence limit ratios (CLRs) used as measures of precision were much larger. As was the case for BSAFs and $_{socw}$s, only small reductions in the uncertainty of Method 2 BAF predictions were gained using sediment sample sizes larger than about 6. Once the number of samples exceeded about 6, the reductions in BAF prediction CLRs become incrementally much smaller. This was the case even when the variability of chemical concentrations in sediment was large. Depending upon the requirements for predictive BAF uncertainty, exceeding sample sizes of 10 appears to be warranted only for sites having very high variability in chemical concentrations in sediment.

Within-chemical correlations were found to be mildly helpful in terms of reducing the uncertainty of Method 2 BAFs; on average, a rank correlation coefficient of 0.5 reduced the CLRs by 21%. Within-media correlation, especially the correlation between chemical concentrations in sediment, significantly reduced the uncertainty of Method 2 BAF predictions when few sediment samples are collected. When only two sediment samples are used to calculate the BSAF and $_{socw}$, a rank correlation coefficient of 0.5 reduced the CLR by 50% in

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comparison to the uncorrelated simulation. Overall, concentration correlations were found to be helpful in terms of improving the precision of Method 2 BAF predictions; this was especially the case when relatively few samples were drawn from sediment concentrations that were correlated between chemicals.

4.4.4 How Can These Methods Be Used to Help Design a Sampling Plan?

The investigator can combine the proceeding two approaches to design a sampling plan to collect the data necessary to predict a BAF of defined accuracy and precision using Method 2. The illustrative sampling structures from Section 4.4.2 (Table 4-1) suggest the number and spacing of sampling events for a field study necessary to determine an unbiased BAF, but not the necessary number of samples to collect. On the other hand, the total number of samples that the investigator should collect in order to obtain a desired BAF can be estimated using the results of the Monte Carlo simulations presented in Section 4.4.3. EPA recommends that the results of modeling simulations be used together with statistical methods such as Monte Carlo analysis as

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the basis for a rational sampling design process. The design process is outlined below:

1. The investigator determines the goal for precision of the BAF prediction, and expresses this goal as the 90% CLR.

2. The investigator selects an appropriate sampling design structure. Guidance is offered in Section 4.5.1 based on:

    a. Chemical factors: hydrophobicity (log K_{ow}) and rate of metabolism; and

    b. Temporal variability of water concentrations, based upon factors of the waterbody at the site.

    Table 3-2 illustrates the relationship between categories of waterbodies (lakes and reservoirs, estuaries and tidal rivers, rivers and streams) and the degree of temporal variability in concentrations observed for various chemicals. The coefficient of variation (CV) for the chemical concentrations generally increase as one moves from quiescent waterbodies towards those that are more advective (flowing) with shorter hydraulic residence times. Therefore, if site-specific data are not available, the investigator can use the waterbody categories in Table 3-2 to estimate the temporal variability of water concentrations.

3. The investigator determines the number of biota, sediment and water samples to collect. Guidance is offered in Sections 4.4.3 for required sample sizes:

    a. If site-specific data or data representative of the site and chemical are available, the investigator should consider conducting Monte Carlo simulations, using concentration moments for the site, to determine sample numbers.

    b. Unless reduced precision and increased bias are acceptable, the investigator should avoid collecting fewer than 6 samples of each medium. These samples

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may be composited within each medium, without reducing the precision of the results (see guidance offered regarding sample compositing in Sections 3.3.5, 3.4.5 and 4.6.4).

4. The investigator allocates the number of samples (based upon guidance from Step 3) evenly among sampling events (determined in Step 2).

4.5 MEASURING CHEMICAL CONCENTRATIONS IN SEDIMENT

This section provides guidance on the development of a field plan for sampling sediment to support the determination of a BSAF for a site. This guidance is based upon a number of documents, including WDNR (1998), USEPA (2001), USEPA (1995) and Versar (1982). These documents provide more detailed guidance on the sampling design of field studies, and recommend field procedures for collecting, preserving, and shipping sediment samples to a processing laboratory for chemical analysis. Planning and documentation of all field procedures should be emphasized to ensure that collection activities are cost-effective and that sample integrity is preserved during all field activities. The investigator should follow EPA's Data Quality Objectives (DQO) process as a recommended systematic planning tool. The information compiled in the DQO process is then used to develop a project-specific Quality Assurance Project Plan (QAPP) which should be used to plan the sediment sampling plan.

The investigator and field sampling staff should develop a detailed sampling plan prior to initiating a field study. For sediment sampling, there are four major parameters that should be specified prior to the initiation of any field sampling activities:

- Target analytes and analytical methods (including ancillary measurements)
- Sampling locations and depth
- Sample type and collection method
- Replicate and composite samples

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The role of each of these parameters in developing an appropriate field plan for sediment sampling is discussed below.

Unlike biota and water sampling, the timing of collection is usually not a significant factor for sediment sampling. When properly sampled, sediments provide time-stable measures of concentrations of persistent bioaccumulative chemicals in aquatic systems. Therefore, sediment sampling can be conducted at a time that is convenient to the field study. The important exception is that sediment sampling should not be conducted immediately after a major disruption in the ecosystem, (e.g., severe flooding, chemical spills, dam removal, lock replacements or dredging operations). At such times, the chemical concentrations in surficial sediment may not be representative of the sediments to which the resident organisms have been exposed. Ecosystems adjust fairly quickly to sediment disruptions, and a year or two is generally sufficient time to allow chemical concentrations in the ecosystem to adjust to the new conditions.

4.5.1 Target Analytes and Analytical Methods

Analytical method(s) used to measure chemical concentrations in sediment must be compatible and consistent with the methods selected for analysis of biota (Section 3.3.1) and water (Section 3.4.1) samples. BSAFs and $_{socw}$s should only be determined for individual chemicals. In cases where the chemical of interest is a mixture (e.g., PCBs, chlordane), the study design must require that individual chemicals composing the mixture be quantified individually.

The investigator should ensure that the methods chosen to analyze chemicals in sediment is sufficiently sensitive to detect ambient concentrations at the site. Based on the methods chosen, the investigator should then determine the minimum sediment mass and volume required for each sample. This requirement is usually stated explicitly in the description of methods used to analyze chemical concentrations in sediment. Since Method 2 calls for the measurement of multiple chemicals (chemical of interest, plus one or more reference chemicals) in sediment samples, the required sample mass and/or volume may be 2 or 3 times larger than required for the analysis of an individual chemical.

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Organic carbon content should be determined in all sediment samples analyzed for the chemical of interest and reference chemicals. The investigator should also consider other ancillary measurements, which may be helpful in interpreting variations in sediment chemistry in terms of physicochemical, biological, and transport processes. These include grain size, bulk density, percent moisture, sediment oxygen demand (SOD), acid volatile sulfide (AVS), and oil and grease. If sediment cores are collected, radioisotope (i.e., Cs-137 and Pb-210) measurements may be useful in determining the deposition age of individual core sections.

4.5.2 Sampling Locations and Depths

The spatial distribution of chemical concentrations in sediment are often highly variable. Therefore, the investigator should carefully consider and select the appropriate locations for sediment sampling. A key consideration for the investigator faced with determining a BSAF at a site, is that the sediment samples should be reflective of the target organism's recent exposure history. In practice, this means collecting sediment samples within an area defined by the home range of the organism. Depending upon the species, the home ranges are different, and larger fishes tend to have larger home ranges. With information about the home range of the fish, an assessment of where the fish resides relative to the spatial variability in chemical concentrations can be performed. Clearly, where large concentration gradients exist at the field site, extra care should be taken in selecting sediment sampling locations to collect representative samples. Geostatistical methods may be used to help identify optimal sampling locations (Leadon, 2000), if some data are available for the spatial distribution of the chemical in the sediment at the site.

The issue of identifying the home range of an organism is discussed in Section 3.3.2. Once the home range is defined, the investigator should next select a spatial sampling design. Guidance is available from several sources (USEPA, 2002; USEPA, 2001[Table 2-1]) regarding sampling design alternatives that may be appropriate for estimating the mean chemical concentration required for the BSAF. Sampling designs are frequently based upon collecting sediment samples at the same locations where biota are collected. This is not necessarily the best

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approach to selecting sediment sampling locations, because these samples may not reflect the biota's average chemical exposure over the home range.

To measure chemical concentrations that are reflective of the target organism's recent exposure history, it is important to sample the surficial layer of sediment. The thickness of the surficial sediment layer is defined by the rate of sediment deposition, bioturbation or physical mixing processes, and other factors responsible for vertical distribution of sediments and associated chemical contaminants within the bed. If this is done successfully, the sediment samples will be "connected" to the biota in terms of chemical exposure.

EPA recommends that samples of surface sediments should be collected from locations in which carbonaceous sediment, containing the chemical of interest and the reference chemicals, is regularly deposited and is representative of average surface sediment in the vicinity of the organism. When selecting sediment sampling locations, it is important to consider sediment deposition and erosion zones, since grain size and related characteristics (including conventional parameters such as sediment organic carbon and acid volatile sulfide, as well as chemical concentrations) are likely to vary between these two sediment environments. In fluvial (flowing) waterbodies, sediments tend to deposit and accumulate in locations where current velocities are lower (e.g., inside stream bends, in deep pools, above dams or other obstructions). In lacustrine waterbodies, sediments usually accumulate in deeper water. Depositional zones typically contain fine-grained (silt and clay) sediment deposits which tend to have higher organic carbon content. Higher concentrations of hydrophobic chemicals are usually associated with fine-grained sediments. Coarser particle types (e.g., sand and gravel) are usually found in erosional zones. Depending on the target organism, one or the other sediment zone may be favored as a substrate or habitat. However, sediment sampling in erosional zones is not recommended because chemical concentrations measured in the sediments typically found in such locations tend to be unrepresentative of the chemical exposure for most aquatic organisms.

Determining the appropriate depth of sediment to collect during sampling is as important a consideration as properly locating the samples. For the investigator determining a BSAF, it is best to sample the upper-most surficial sediment layer, because this sediment contains the

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chemical concentrations to which fish are exposed, as well as the benthic food web for the fish. Generally, the most recently deposited sediments and most epifaunal and infaunal organisms are found in this surficial layer. The goal for the investigator is to sample a thin layer of surficial sediment. EPA considers the top 1 cm of sediment to be ideal (Burkhard et al. 2004), although for many sites this may be overly conservative as well as impractical in terms of sampling methods and analytical volume/mass requirements. Depending on site-specific factors, it may be acceptable to increase this depth to the upper 2 or 4 cm of sediment. When benthic organisms, especially oligochaetes (small earthworm-like organisms) are abundant, the surficial sediment horizon is often vertically well-mixed by bioturbation to a depth up to about 10 cm (Boudreaux, 1994; Thibodeaux and Bierman, 2003). However, samples containing sediment from deeper in the bed may tend to bias the measurement of chemical concentrations, because the concentrations in deeper sediment intervals are often different than in the surficial sediment. Additionally, if loading histories for some but not all of the chemicals have changed, sediment samples extending to deeper levels might provide skewed representations of the distribution of chemicals. Sediment samples extending from the sediment surface to much deeper levels in the sediments, e.g., 0-20 cm (a common sediment sampling protocol) will in many cases be too deep to be acceptable, and could represent time periods extending to decades or more depending upon sedimentation rates. The investigator should review any available data for the site that can be used to determine the appropriate depth of surficial sediment for sampling. These data include sediment core profiles of chemical concentrations, physical properties or counts of benthic organism abundance. If such data cannot be found, the most conservative approach for the investigator would be to sample the top 1-2 cm of surficial sediment.

A review of existing background information from all reasonably available sources for a site or study area should be the first step in collecting data for a sediment quality assessment. The information obtained in a review of a site's historical (industrial and other uses) and existing sediment data costs relatively little and can provide information about the likelihood and types of contamination that may be present. Historical information can help guide study plans and may reduce the amount of field work and analysis needed to accomplish information goals. Various types of information may be available for a site background review, and the investigator should pursue this data at the project planning stage:

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Historical Information - Historical information is useful in trying to find out what chemical contaminants may have been introduced to the waterbody historically, and can indicate specific contaminants that may be targeted as reference chemicals. Historical information includes:

- Land use - agricultural, industrial, residential, recreational;
- Water usage - industrial, municipal wastewater treatment plants, power plants, municipal water intakes, shipping;
- Dredging activity; and
- River, lake, estuary or harbor morphology and bathymetry.

Recent information - Additional information, generated within the past 10 years, should also be sought, such as:

- Precise description of designated uses;
- Quantity and quality of potential and known inputs;
- Point sources - locations of outfalls from industrial discharges, storm sewers, etc.;
- Non-point sources of sediment and chemical contamination;
- Any previous sampling and chemical analysis data;
- Sediment (bathymetric) maps - Many harbors have up-to-date bathymetric maps of the harbor area. The local harbor authority, U.S. Army Corps of Engineers (USACE), U.S. Coast Guard, or National Oceanic and Atmospheric Association (NOAA) should be able to provide that information.

From this historical information, the investigator may be able to develop an understanding of the following factors affecting contaminant source pathways: bathymetry, water current patterns, tributary flows, watershed hydrology and land uses, sediment and soil types, and sediment deposition rates. Assembling this information can be helpful in evaluating

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sampling locations and sample designs (i.e., the choice of sampling on a regular grid or stratified random sampling). The following are suggested sources of information relevant to sediments and chemical contamination:

- STORET (http://www.epa.gov/storpubl/) - A database maintained by EPA to store and make available data on many water quality parameters, including contaminant concentrations in sediment and fish.
- NWISWeb (http://waterdata.usgs.gov/nwis) - Database maintained by US Geological Survey, also including contaminant concentrations in water and sediment for major tributaries.
- Sediment and Fish Contaminant Databases, e.g.:
    - USEPA Ecotox,
    - USEPA Fish Residue,
    - USEPA PCB Residue,
    - USACE Residue and other studies of sediment pollution and sediments.
- Published scientific research - A search of the published literature (mostly journals) should be conducted for any research that has been conducted at the study site.
- Published and unpublished reports - Studies may have been carried out by states and reported, but never formally published. These reports may contain valuable information about sediment sites.
- Case files - Many states maintain files containing information and reports on previous and ongoing remediation projects. EPA studies and information about Superfund and RCRA sites, Remedial Action Projects (RAPs), basin plans, Total Maximum Daily Load (TMDL), and NPDES permit records may also be contained in the State case files.
- Local government or academic related research - Local health agencies, Fish and Wildlife Service, and colleges and universities (natural resources, environmental chemistry and environmental science and engineering programs) may be excellent resources and sources of information.
- Selected chemical spill reporting system (EPA) - Information is available from the states and directly from the EPA.

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- Pesticide spill reporting system (EPA).
- Reports of pollution-caused fish kills.
- Pollution incident reporting system (U.S. Coast Guard).
- Identification of In-Place Pollutants and Priorities for removal (EPA).
- Hazardous waste sites and Management facilities reports (EPA).
- U.S. Army Corps of Engineers (ACE) studies of sediment pollution and sediments.

4.5.3 Sample Type and Collection Method

Sediment samples are most commonly collected using a coring device or a dredge or grab sampler. The type of collecting equipment chosen will depend on sediment texture, site location (depth and current velocity), analyses to be performed, and study goals. Guidance in selecting appropriate sediment sampling equipment is provided in Chapter 3 of USEPA (2001). The technical manual includes flowcharts for selecting appropriate core and grab samplers based on site-specific factors.

A piston corer allows excellent quantitative and qualitative sampling to a specified sediment depth with little disturbance of the sediment-water interface. Samples can be separated or stratified by depth or color/texture to analyze distinct layers of sediment, although the sediment along the side of the core may smear as the core penetrates, slightly distorting the stratification of the sediment. A corer may not be able to penetrate or retain very sandy substrates. Coring in high clay-content sediments where grab samplers won't work is possible if the water is not too deep, but may be difficult with a push corer and may require the use of a slide hammer or vibrating corer. A hand-operated, 3 inch diameter core sampler with an optional piston and extensions for deeper water can be effectively used in soft sediments with some silt/clay content in water up to ~30 ft deep. A large bore corer will provide a larger volume of sediment per attempt. This is important if discrete sample replicates are desired. Even with the large bore core tube, samples may need to be composited to obtain enough sediment volume for the required chemical analyses.

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Grab samplers rely on their own weight and gravity to penetrate the sediment as well as the leverage from the closing of the jaws. For this reason, they are not as efficient in water flowing with a velocity over one meter per second. They normally take a discreet "bite" of sediment to a fairly consistent and measurable depth. Grabs often cause a shock wave upon descent which may disturb very fine sediment at the sediment-water interface. Common grab samplers include the petite Ponar and Ekman dredges, both of which can be hand operated from a small flat-bottomed boat. The Ponar is better suited to sampling hard or sandy sediments because of the greater ability to penetrate, while the Ekman is more suited to sampling in soft sediments in low flow waters. Neither grab sampler will effectively sample hard clays; a coring device or shovel such as a sharpshooter spade should be used at these sites.

4.5.4 Replicate and Composite Samples

As discussed in Section 4.4, the number of samples directly affects the representativeness and completeness of the sediment data for estimating the mean chemical concentration. The number of samples collected and analyzed will always be a compromise between the desire of obtaining high quality data and the constraints imposed by analytical costs, sampling effort and logistics. The investigator can use two strategies to find an appropriate balance between confidence in the data and cost of collecting it: replication and sample compositing.

Sample replication is used to assess measurement precision, and can be used to determine the variability in data due to analytical errors and sampling reproducibility, factors which can be significant in comparison to the spatial variance in chemical concentrations. Different kinds of replicates can be collected, depending on the type of precision desired by the investigator. Analytical replicates are used to assess analytical data quality. Field replicates can be used to provide useful information on the heterogeneity of chemical concentrations within sediment, for either the site or for locations (stations) within the site. Results of field replicate analysis yield the overall (combined) variability or precision of both the field and laboratory operations. This variability is an important factor in estimating the minimum number of sediment samples necessary to determine a BSAF or Π_{socw} of known precision, as discussed in Section 4.4.3. When collecting replicate samples to statistically compare sediment deposits, sample sites within each

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deposit should be randomly located for statistical comparisons to be valid. Each replicate sample should be taken from an area of sediment undisturbed by previous samples.

A composite sample is formed by combining material from more than one sample or subsample. Because a composite sample is a combination of individual aliquots, it represents an average of the characteristics making up the sample. Although compositing results in a less detailed description of the variability in chemical concentrations, it is generally considered an excellent way to average the naturally heterogeneous physical and chemical conditions in sediment that often exist at a site, even within a relatively small area. Compositing is also a practical way to control analytical costs while still providing a reliable mean chemical concentration based upon samples from a large number of locations. Compositing of sediment samples is not recommended where combining samples could serve to dilute a highly-concentrated but localized sediment "hot spot". Also, sediment samples from locations with very different grain size characteristics or different stratigraphic layers of core samples should not be composited. Multiple grabs or cores for a composite sample should be taken from a relatively homogeneous sediment deposit (i.e., all grabs should be of similar sand/silt content).

In some cases, composite samples are needed to generate sufficient sample volume for all analyses. This is particularly true when sampling a relatively thin layer of surficial sediment. Table 4-2 shows the number of 3-inch core samples that must be composited to generate 500 mL of sediment, a common volume requirement for analysis of multiple organic chemicals. As this table illustrates, an increasing number of samples must be composited to obtain a required sediment volume, as the thickness of the surficial sediment being sampled decreases.

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Table 4-2. Number of 3-inch Diameter Cores Required to Composite 500 mL of Sediment

surficial sediment sample thickness (cm)	volume per core (cm^3)	number of cores required to obtain 500mL volume
1	45.6	11
2	91.2	6
4	182	3
10	456	1

4.6 SCIENTIFIC ISSUES ASSOCIATED WITH METHOD 2 AND THE USE OF BSAFs TO PREDICT CHEMICAL BIOACCUMULATION

EPA's Method 2 bioaccumulation methodology has not received widespread attention in either the scientific or regulatory communities. Nor does EPA have much experience in the application of Method 2 to predict site-specific BAFs. Consequently, there are many potential issues to address dealing with the reliability of Method 2 predictions and the underlying assumptions upon which it is based. This section discusses a number of significant scientific issues related to the application of Method 2.

4.6.1 Evaluation of Method 2 Predictions of Site-Specific BAFs?

A number of studies have been conducted to evaluate the prediction of BAFs by Method 2, using BSAFs measured at a site. Evaluation efforts have been conducted with data collected from three aquatic ecosystems in the United States: Lake Ontario; Green Bay/Fox River, Wisconsin; and the Hudson River, New York. EPA previously published information on evaluation of the Method 2 approach by using data on PCBs, chlorinated benzenes, pesticides, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) collected from Lake Ontario and the mid-bay region of Green Bay (USEPA, 1995c). Baseline BAFs for PCBs, chlorinated benzenes, and some

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pesticides were predicted from BSAFs for Lake Ontario salmonids and compared with measured baseline BAFs from the same system. The baseline BAFs predicted from BSAFs were within a factor of 4 of the measured baseline BAFs. Furthermore, when predicted baseline BAFs for TCDD and PCBs from Green Bay brown trout and Lake Ontario salmonids were compared, the baseline BAFs predicted from BSAFs were generally within a factor of 2 of the measured baseline BAFs. Although there were a few outliers in the observed trends, the results of this evaluation effort showed Method 2 generally works well, not only for predicting baseline BAFs with data from the same ecosystem (Lake Ontario), but also for predicting baseline BAFs between systems (Green Bay vs. Lake Ontario).

Burkhard et al. (2003b) extended the previous evaluations for Method 2 by comparing results of field-measured baseline BAFs with baseline BAFs predicted from BSAFs using additional PCB data collected from Green Bay/Fox River and the Hudson River. The data sets for this latest evaluation effort were selected from the 1989–1990 Green Bay Mass Balance Study (http://www.epa.gov/grtlakes/gbdata) and the Hudson River PCBs Reassessment Remedial Investigation/Feasibility Study (USEPA, 1998). The former study included data from the lower Fox River and the inner, middle, and outer zones of Green Bay. The Hudson River data were collected over several years by a number of federal and state agencies and private groups and were assembled into a single database (USEPA, 1998) from which data were selected for this analysis. The reference PCB congeners used in this evaluation effort included three of those used in the previous validations (PCB 52, 105, 118) (USEPA, 1995b) as well as PCBs 18, 28, 149, 174, and 180. This evaluation was performed using the geometric mean of the baseline BAFs predicted by using as many reference chemicals as possible from the eight PCB congeners listed above. EPA recommends that several reference chemicals be used with Method 2 and that K_{ow}s be matched as closely as possible, because slightly smaller predictive errors were observed in the evaluation study when the chemicals of interest and the reference chemicals had more closely matched K_{ow}s (Burkhard et al. 2003b). The evaluation effort by Burkhard et al. (2003b) included baseline BAFs for several fish species in addition to salmonids (e.g., carp, walleye, shad, alewife, yellow perch, white perch, pumpkinseed, red-breasted sunfish, and largemouth bass), some of which spanned several age classes.

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A summary of the evaluation exercise is presented here and a detailed discussion is provided by Burkhard et al. (2003b). Baseline BAFs predicted with Method 2 were plotted against field-measured baseline BAFs, to visually demonstrate the accuracy and precision of the predictions. The agreement between measured and predicted $BAF_{i,L}^{fd}$ s using Method 2 is illustrated in Figure 4-2 for Green Bay and the Hudson River, for a variety of the fish species. The ratio of predicted-to-measured congener-specific baseline BAFs ($BAF_{predicted}/BAF_{measured}$) was used to evaluate the agreement between Method 2-predicted baseline BAFs and field-measured baseline BAFs. Table 4-3 presents zone (Green Bay data) and location-specific (Hudson River data) statistics for the $BAF_{predicted}/BAF_{measured}$ ratio. Table 4-3 also presents the percentage of $BAF_{predicted}/BAF_{measured}$ ratios that fall within specified ranges of the distribution. In general, the agreement between Method 2-predicted baseline BAF and field-measured baseline BAF values is very good, with a majority of predicted BAF values falling within a factor of 2 of the field-measured BAF values. In addition, >90% of Method 2-predicted BAFs (94.5% from Green Bay and 90.7% from Hudson River) are within a factor of 5 of the field-measured baseline BAFs. For most zones in Green Bay, the 95% exceedance levels (i.e., 95% of the $BAF_{predicted}/BAF_{measured}$ values) fall within the range of 0.2 (one-fifth of the predicted baseline BAF) to 5.0 (five times the predicted baseline BAF). Results for the Hudson River indicated generally similar agreement between Method 2-predicted baseline BAFs and field-measured baseline BAFs. Overall, these analyses strongly support the use of Method 2 to estimate site-specific BAFs from field-measured BSAFs.

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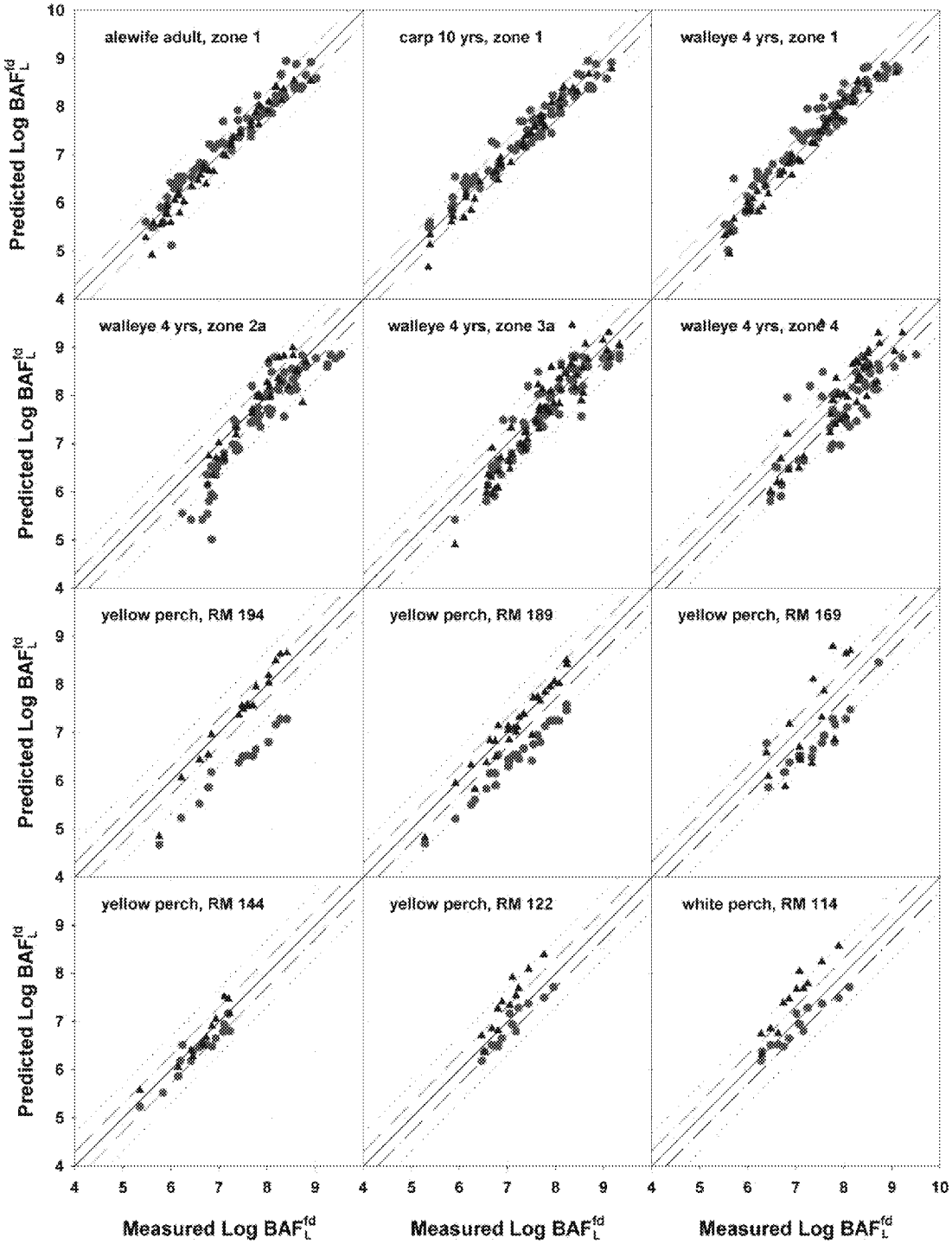

Figure 4-2. Predicted baseline BAFs using method 2 (blue triangle) and 4b (pink circle) plotted against measured baseline BAFs for different sampling locations and fish species for the Green Bay (species and zone) and Hudson River (species and river mile) ecosystems. The solid lines are perfect (1:1) agreement, the dot-long dash lines are 2x from perfect agreement, and the dotted lines are 5x from perfect agreement.

Table 4-3. Validation Statistics for Method 2: Ratio of Baseline $BAF_{predicted}$/Baseline $BAF_{measured}$ for all (combined) sampled fish species

Location	Method 2: Exceedance Levels and Comparison Statistics					
	95%	Mean	Median	5%	% within 2x	% within 5x
Green Bay						
Zone 1	0.39	0.88	0.88	1.66	87.6	100
Zone 2a	0.25	1.27	0.89	3.47	69.8	92.8
Zone 3a	0.21	1.25	0.73	3.78	51.5	94.1
Zone 3b	0.16	1.08	0.69	2.71	53	91.4
Zone 4	0.31	3.33	1.07	3.79	31.9	97.4
All zones	0.22	1.53	0.81	3.29	55.7	94.5
Hudson River						
RM 194	0.46	1.12	0.99	2.12	81.9	95.2
RM 189	0.33	1.00	1.03	1.55	87.5	100
RM 169	0.11	2.01	0.59	9.91	19.0	68.3
RM 144	0.67	1.19	0.97	2.14	92.3	100
RM 122	0.70	2.43	2.16	4.81	45.8	95.8
RM 114	1.20	3.86	3.78	6.91	16.7	83.3
All stations	0.13	1.50	1.10	4.42	64.9	90.7

RM = river mile.

The agreement between Method 2 BAF predictions and measurements was less satisfactory in Green Bay zone 4 and at Hudson River RM-114. In both ecosystems, these locations are relatively distant from the major known sources of chemical contamination. As PCBs are transported greater distances, they are increasingly subject to various transport and fate processes which can alter their concentrations and concentration ratios (i.e., the "weathering" process: Burkhard et al., 1985; Mackay et al., 1992; Manchester, 1993). PCB concentrations are lower at these "distant" locations than in other zones/river stations closer to the major known sources. Lower chemical concentrations are generally less accurate than higher concentrations,

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which may lead to greater errors in BAF predictions. In addition, other sources (e.g., atmospheric deposition) may become more significant contributors of PCBs at these distant locations. Each of these factors may play some role in the poorer fit of the Method 2 BAF predictions to measurements made at distant locations.

4.6.2 Is Chemical Equilibrium Assumed in the Calculation of a BSAF?

The BSAF definition (equation 4-1) does **not** invoke or include the assumption of equilibrium conditions for the chemical between the organism and sediment (Ankley et al., 1992; Thomann et al., 1992). As shown by Thomann et al. (1992), BSAFs are appropriate for describing bioaccumulation of sediment contaminants in aquatic food webs with non-equilibrium conditions between both the sediment and fish, and sediment and its overlying water. Equilibrium is regarded as a reference condition for describing degrees of disequilibrium between chemical concentrations in biota, sediment and water. Therefore, chemical equilibrium is not a requirement for measurement, prediction, or application of BSAFs.

When calculating BSAFs for benthic invertebrates, numerous investigators (Lake et al. 1984; McElroy and Means, 1988; Bierman, 1990; Lake et al. 1990; Ferraro et al. 1990) have invoked two assumptions: (1) equilibrium conditions and (2) no metabolism of the chemical. These assumptions, when combined with EqP (equilibrium partitioning) theory (DiToro et al. 1991), lead to the conclusion that the BSAF, for these specific conditions, is equal to the partitioning relationship of the chemical between organic carbon in the sediment and lipids of the organism. Depending upon the affinities of the nonpolar organic chemical for lipid and sediment organic carbon, the BSAF, under these specific conditions, should be in the range of 1 to 2 (McFarland and Clarke, 1986.). For aquatic organisms tightly connected to the sediments like oligochaetes and other benthic invertebrates, experimental measurements (Lake et al. 1990; Tracy and Hansen, 1996) are generally consistent with the theoretical value, i.e., in the range of 1 to 2.

These data show that chemical equilibrium is a sound fundamental theory for nonionic organic chemicals when appropriately applied to conditions near equilibrium.

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However, there are solid mechanistic reasons why fish should not be in equilibrium with sediments within their home range (Thomann et al. 1992). For fish, BSAFs incorporate wide ranges of influences including: biomagnification due to the trophic level of the fish, sediment-water column chemical disequilibrium, the diet of the fish and its underlying food web, the fish's home range, and chemical metabolism within the fish and its food web (Burkhard et al. 2003a). Suggestions that BSAFs for fish should be in the range of 1 to 2 by combining the definition of the BSAF with the assumptions of equilibrium conditions and no metabolism are incorrect (Wong et al. 2001). Due to these factors, measured BSAFs with values above or below 1 to 2 are entirely reasonable for fish (Burkhard et al. 2003a). BSAFs outside of this range for fish do not violate the general definition of BSAFs nor invalidate the usefulness of BSAFs in predicting chemical residues in fish for sediment contaminants.

For BSAFs to have predictive power in terms of determining BAFs (i.e., Equation 4-2), the ratio of chemical concentrations between biota and sediment should not change substantially over time. This implies that the site is at or near steady state conditions for the chemical of interest and the reference chemicals. The parameter $D_{k/r}$ offers the investigator some ability to correct for differences in sediment-water concentration quotients (Π_{socw}) that may reflect mild departures from this condition. To reiterate, steady state conditions do not require chemical equilibrium.

4.6.3 Review of Existing Data for $_{socw}$

Reliable measurements of $_{socw}$s are rather limited because of a number of factors. These include :

- the difficulties in measuring the concentrations of hydrophobic organic chemicals in natural waters because they occur at very low concentrations, that is, less than 1 ng/L;
- the lack of data for sediment and water samples that are temporally and/or spatially coordinated;

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- the lack of data for sediment samples collected from the uppermost 1 or 2 cm of the sediments;
- the lack of measurements of POC and DOC for the water samples analyzed for the hydrophobic organic chemicals;
- the lack of determinations of the sediment organic carbon content; and
- the fact that studies designed specifically for determining $_{socw}$ are not usually performed.

In addition, combining sediment measurements from one study with water measurements from another study can result in large biases in $_{socw}$s due to differences in analytical methodologies (e.g., different surrogates for recovery corrections, different standards) and sample designs.

Review of a number of different data sets, as described in Burkhard (1998), revealed three data sets of suitable quality for which $_{socw}$s could be determined. These data sets were from Lake Ontario (Oliver and Niimi, 1988), Hudson River (USEPA, 1997; USEPA, 1998), and Green Bay in the Lake Michigan ecosystem (www.epa.gov/grtlakes/gbdata/). The Green Bay and Hudson River data sets contained data for PCBs only, and the Lake Ontario data set contained data for chlorinated pesticides, PCBs, and a few chlorinated benzenes, toluene, and butadiene. The data for the chlorinated benzenes, toluene, and butadiene in the Lake Ontario data set were not used in this analysis because these chemicals volatilize to the atmosphere relatively easily in comparison with the higher molecular weight PCBs and chlorinated pesticides.

Figure 4-3 shows the $_{socw}$s for selected PCB congeners in five different zones of Green Bay. For the individual PCB congeners, the geometric mean regressions were performed on data for the five different zones in the Green Bay system because both variables were measured with error (Ricker, 1973). The slopes of the log $_{socw}$-log K_{ow} regressions from the different zones were not significantly different among the five zones (comparison of slope test, = 5%). Therefore, average $_{socw}$s were determined for each PCB congener with data from all zones (Figure 4-4). The geometric mean regression statistics are reported in Table 4-4 for each zone and for the average of all zones. Examination of Figures 4-3 and 4-4 and Table 4-4 reveals that

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for PCBs, $_{socw}$ is strongly dependent on the K_{ow}, and slopes of slightly less than 1 were obtained. Examination of $_{socw}$s for Lake Ontario and Hudson River reveals trends similar to those in Green Bay; a strong dependence of $_{socw}$ on K_{ow} for the PCBs and chlorinated pesticides (Figure 4-5 and Table 4-4), and slopes of 1 and slightly less than 1 were obtained.

Table 4-4. Geometric Mean Regression Equations (log $_{socw}$ = A log K_{ow}+ B) for Polychlorinated Biphenyls (PCBs) and Chlorinated Pesticides

Ecosystem	Slope (±sd)	Intercept (±sd)	n	r	s_{xy}
Green Bay (PCBs)					
Zone 1	0.95 (±0.04)	1.21 (±0.22)	46	0.97	0.17
Zone 2a	0.92 (±0.09)	1.13 (±0.61)	31	0.82	0.34
Zone 3a	0.87 (±0.06)	1.61 (±0.36)	63	0.86	0.37
Zone 3b	0.83 (±0.06)[a]	1.88 (±0.36)	60	0.85	0.33
Zone 4	0.86 (±0.08)	1.31 (±0.53)	46	0.76	0.46
All zones, congener averages	0.92 (±0.06)	1.20 (±0.38)	77	0.82	0.43
Hudson River (PCBs)					
RM 189	0.87 (±0.08)	1.81 (±0.45)	32	0.86	0.13
RM 194	0.72 (±0.08)[a]	3.16 (±0.42)	27	0.84	0.16
Lake Ontario (PCBs and chlorinated pesticides)	1.05 (±0.08)	0.83 (±0.49)	55	0.84	0.46

n = number of data points

r = correlation coefficient

sd = standard deviation

s_{xy} = standard error of estimate

[a] slope significantly different from 1.0, = 1%.

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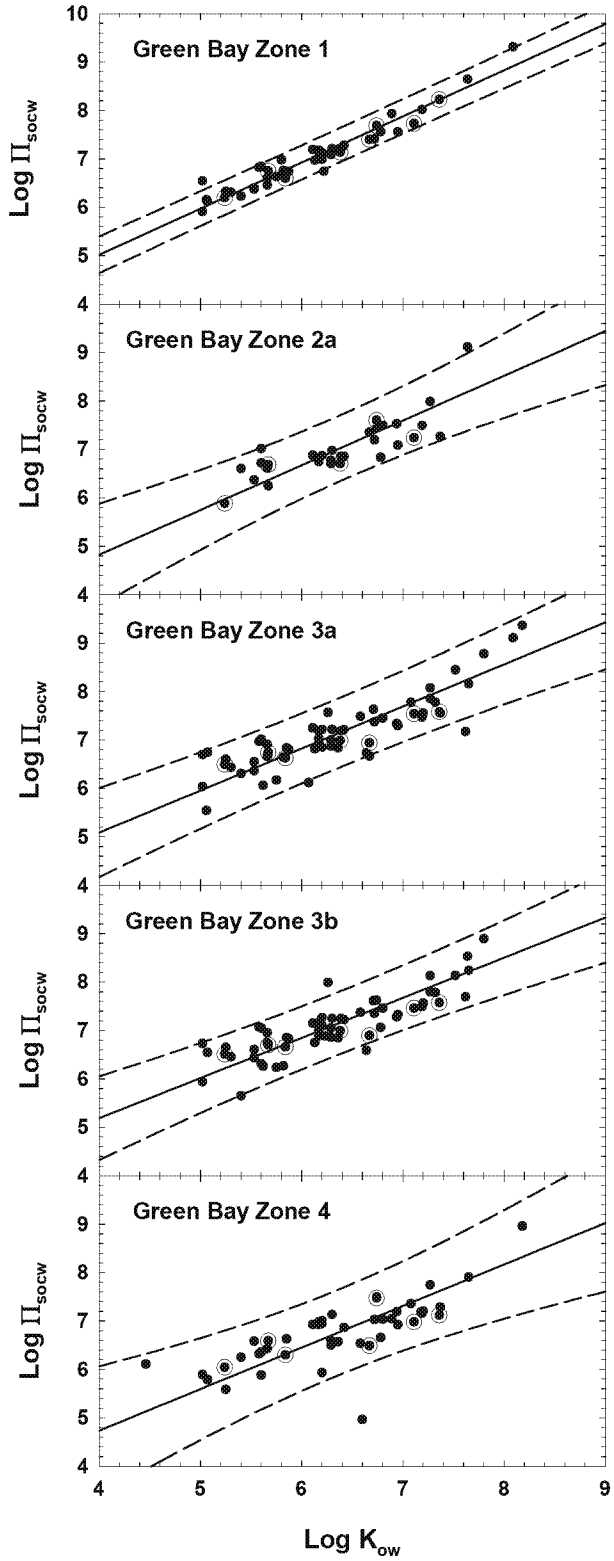

Figure 4-3. Sediment-water column concentration coefficient ($_{socw}$) for PCBs in five different geographical zones in Green Bay, Lake Michigan. The circled data points are the PCB congeners numbers (log K_{ow}) 18 (5.24), 28 + 31 (5.67), 52 (5.84), 101 (6.38), 118 (6.74), 149 (6.67), 174 (7.11), and 180 (7.36). The geometric mean regression and their 95% confidence limits are plotted.

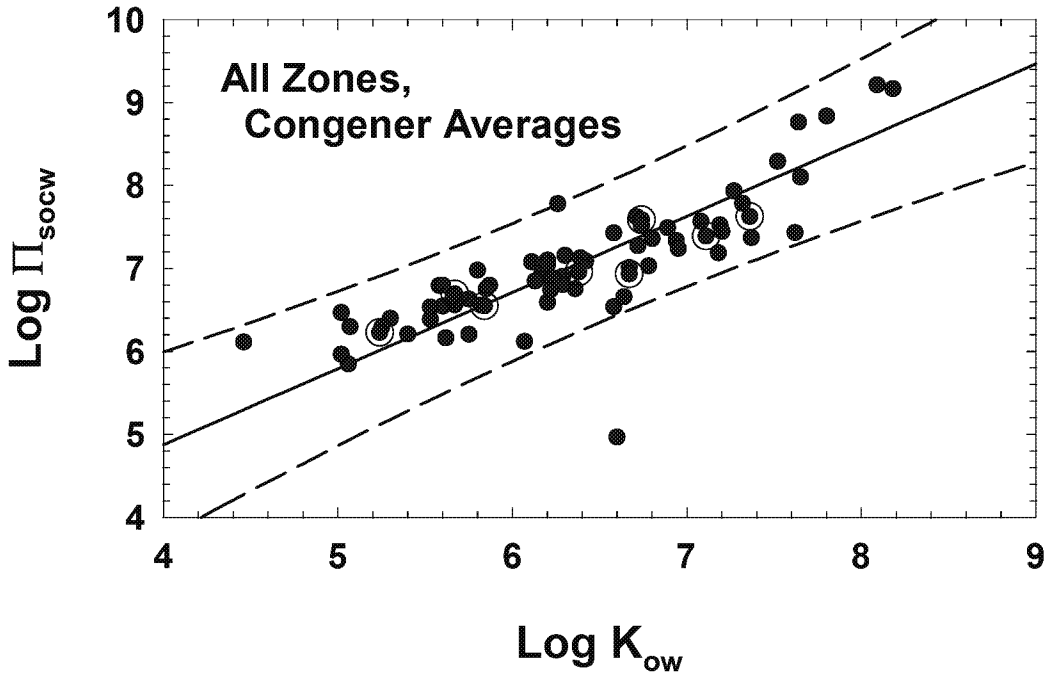

Figure 4-4. Average sediment-water column concentration coefficients ($_{socw}$) for individual PCB congeners across the five different geographical zones in Green Bay, Lake Michigan. The circled data points are the PCB congeners numbers (log K_{ow}) 18 (5.24), 28 + 31 (5.67), 52 (5.84), 101 (6.38), 118 (6.74), 149 (6.67), 174 (7.11), and 180 (7.36). The geometric mean regression and their 95% confidence limits are plotted.

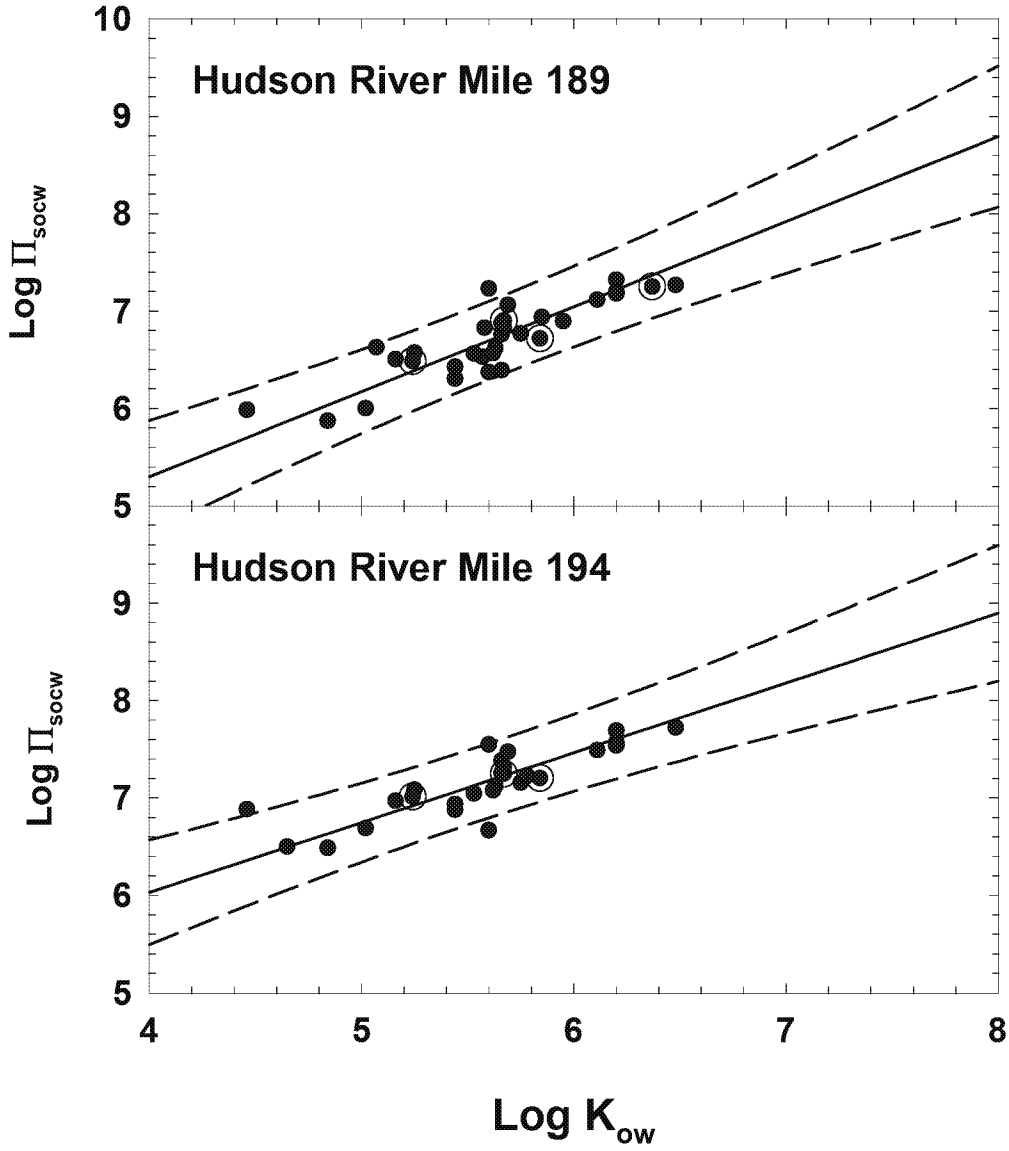

Figure 4-5. Sediment-water column concentration coefficients ($_{socw}$) for PCBs at river miles 189 and 194. The circled data points are the PCB congeners numbers (log K_{ow}) 18 (5.24), 28 + 31 (5.67), 52 (5.84), 101 (6.38), 118 (6.74), 149 (6.67), 174 (7.11), and 180 (7.36). The geometric mean regression and their 95% confidence limits are plotted.

In the Green Bay ecosystem, chemical concentrations in both sediments and the water column decrease with increasing zone number. Zone 1 is at the mouth of the Fox River, the source of PCBs to the bay, and zone 4 connects the bay to Lake Michigan. Zone 1, the region of highest chemical concentrations, has much less variability in the measured $_{socw}$s and the largest slope for the log $_{socw}$–log K_{ow} relationship among all sampling zones in Green Bay. Comparison of the variability existing in zones 1 through 4, as illustrated by the 95% confidence intervals in Figure 4-3, suggests that variability increases with increasing distance from the source of the PCBs (Table 4-4), and this trend parallels the concentration gradient in Green Bay. The consistency and slope of the $_{socw}$–K_{ow} relationships observed in zone 1 data might be more illustrative of the underlying $_{socw}$–K_{ow} relationships than those of the other zones because of lower uncertainties associated with the analytical measurements, the chemical concentrations are high (i.e., concentrations well above the quantitation limits or high signal-to-noise ratio). This can be explored by data visualization methods (e.g., plotting BAF and/or BSAF variability against water, sediment and biota concentrations). Alternatively, the difference may reflect the greater role that various transport and fate processes (which depend on chemical-specific factors of solubility, volatility and resistance to biochemical degradation) play in outer regions of Green Bay as opposed to their much more limited role nearer the sources of contamination in the Fox River.

From a theoretical standpoint, log $_{socw}$–log K_{ow} relationships will have a slope of 1 if the ecosystem is at equilibrium. In addition, EPA believes that ecosystems at steady state or with conditions that approximate the longer term average conditions will also have slopes nearly equal to 1. A number of factors could cause the slope to be less than 1; these include volatilization losses (assuming net water-to-air flux for all chemicals) although this requires information about the relative volatility of the different chemicals (Mackay et al., 1992), inaccuracies in the calculation of the concentration of chemical that is freely dissolved in the water column (the denominator in the $_{socw}$ term), and measurement error in determining the concentrations of chemical in the sediments and/or water column. The log $_{socw}$–log K_{ow} relationships for the Hudson River, Lake Ontario, and Green Bay ecosystems have slopes that are 1 or slightly less than 1 for PCBs and chlorinated pesticides (Table 4-4). The smallest slopes were observed with the Hudson River ecosystem data. The Hudson River ecosystem is much

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more dynamic and possibly further from steady-state conditions than are the Lake Ontario and Green Bay ecosystems, because of changing flows over time and recent changes in PCB loadings. Given the similarity in slopes among all three ecosystems, the conditions in the Hudson River do not appear to be greatly different from those in the other two ecosystems.

Given that the slopes for the log $_{socw}$–log K_{ow} relationships in Green Bay, the Hudson River, and Lake Ontario are close to 1, and the fact that ecosystems tend to move toward the theoretical slope of 1 over time, EPA assumes a slope of 1 for this relationship. Data analyses and averaging performed for the three ecosystems yielded average $_{socw}/K_{ow}$ ratios of 7.21 for Green Bay, 14.3 and 48.4 for Hudson River, and 23.4 for Lake Ontario (Table 4-5). The large differences in average $_{socw}/K_{ow}$ ratios between the two Hudson River sampling stations suggest distinctly different behaviors in the two sampling stations, and, therefore, an overall ratio was not computed for the Hudson River. The EPA believes that the differences in average $_{socw}/K_{ow}$ ratios among the three ecosystems evaluated here illustrate the range of variability that occurs among ecosystems across the nation. Because $_{socw}$s are a function of both current and past chemical loadings to the ecosystems, $_{socw}/K_{ow}$ ratios both larger and smaller than those observed probably exist in the nation. For highly contaminated sites (e.g., Superfund sites with large concentrations of chemicals in the sediments), $_{socw}/K_{ow}$ ratios could become very large. For new chemicals that are just being introduced or discharged into the environment, $_{socw}/K_{ow}$ ratios will be small because very little of the chemical is present in the sediment. Degradation processes such as hydrolysis, photolysis, and metabolism can also strongly influence the $_{socw}/K_{ow}$ ratio, depending on where these processes occur (i.e., the sediment and/or the water column).

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Table 4-5. Average $_{socw}/K_{ow}$ Ratios for Three Different Ecosystems

Ecosystem	Average Ratio (±sd)	Percentile 5%	10%	90%	95%
Green Bay (PCBs)					
Zone 1	9.15 (±4.97)	4.34	5.55	13.8	17.3
Zone 2a	6.35 (±6.73)	1.24	1.37	13.1	21.0
Zone 3a	10.3 (±13.3)	1.27	1.88	21.7	25.6
Zone 3b	9.48 (±10.6)	1.68	2.00	20.1	29.9
Zone 4	4.49 (±6.68)	0.60	0.75	6.95	8.10
All zones, congener averages	7.21 (±6.68)	1.01	1.76	13.3	16.5
Hudson River (PCBs)					
RM 189	14.3 (±8.98)	6.03	7.36	23.4	34.7
RM 194	48.4 (±47.6)	18.9	22.6	69.5	83.6
Lake Ontario (PCBs and chlorinated pesticides)	23.4 (±25.1)	2.96	3.57	52.6	82.4
Overall average $_{socw}/K_{ow}$	23.3 (±18.0)				

sd = standard deviation

Because the degradation rates for the observed PCBs and chlorinated pesticides in the environment are extremely slow, the average $_{socw}/K_{ow}$ ratio of 23.3 for the three ecosystems is representative of chemicals that are very slowly degraded (or have long half-lives in the environment). Chemicals with higher degradation rates will, in all likelihood, have $_{socw}/K_{ow}$ ratios that are different from those for the PCBs and chlorinated pesticides, and EPA believes that the $_{socw}/K_{ow}$ ratios will be smaller for such chemicals, on average, than those for the PCBs and chlorinated pesticides reported here.

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4.6.4 How does $_{socw}$ Reflect Steady State Conditions at a Site?

Bioaccumulation of hydrophobic nonionic organic chemicals in aquatic organisms is dependent on a number of ecosystem conditions including food chain length (Rasmussen et al. 1990), food web composition (Vander Zanden and Rasmussen, 1996; Burkhard, 1998), and the chemical distribution between sediments and water (Thomann et al., 1992; Endicott and Cook, 1994). The impacts of food web composition and chemical distribution between sediments and water are interrelated because sediments and water are the primary exposure media for the benthic and pelagic components, respectively, of the food web (Burkhard, 1998). Chemical concentrations in benthic invertebrates at the base of the benthic food web are directly controlled by the concentrations of chemicals in the sediments. Chemical concentrations at the base of the pelagic food web (e.g., phytoplankton) are directly controlled by the concentration of chemicals in the water. Therefore, differences in distribution of chemical between sediment and water, as well as differences in benthic versus pelagic food web composition, will affect the bioaccumulation of nonionic organic chemicals in forage and piscivorous fish.

Ecosystems at thermodynamic equilibrium, a condition that rarely exists in nature, should theoretically have $_{socw}$s equal to the chemical's K_{ow}. Consequently, ecosystem models typically characterize $_{socw}$ by using its ratio to K_{ow} as a measure of the degree to which the ecosystem is in disequilibrium (Thomann et al., 1992), or, alternatively, as a measure of the fugacity ratio (Campfens and Mackay, 1997). A $_{socw}/K_{ow}$ ratio of 1 is equivalent to equilibrium conditions between the sediments and the water column. A ratio of 25, which has been typical of Lake Ontario conditions for PCBs and DDTs since the 1970s, is a disequilibrium condition in which the chemical is enriched in the sediments relative to the water column because of greater loadings of the chemical to the ecosystem in the past. For ratios less than 1, the chemical is enriched in the water column relative to the sediments; in this situation, the aquatic ecosystem is being loaded with the chemical, but sediments have not reached steady state with the water ($_{socw}$ constant). With continued loading, sediment contamination increases until a steady-state condition is reached ($_{socw}$ constant) and the $_{socw}/K_{ow}$ ratio is in the 2–10 range. The lower bound of 2 arises from minimum expected differences in the organic carbon content of particulate matter in the water column and sediments. The upper bound of 10 allows for the

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effects of chemical gradients and greater relative organic carbon amounts in the water column. Green Bay, a fairly shallow and vertically well-mixed ecosystem receiving a continuous load of PCBs from the contaminated Fox River in Wisconsin, has a $_{socw}/K_{ow}$ ratio of approximately 5. This ratio indicates that the system is close to steady state and that most or all of the disequilibrium is attributable to differences in organic carbon in the water and sediments.

On the basis of monitoring reports and historical loading data, EPA expects that most persistent nonionic organic chemicals will have $_{socw}/K_{ow}$ ratios in the range of 2-40. This expectation does not apply when such chemicals have not been present in an ecosystem long enough to approach expected steady-state concentrations in surficial sediments. In this case, $_{socw}/K_{ow}$ will be substantially lower than 2, indicating low exposure potential through the benthic food web.

4.6.5 Assumptions and Limitations Associated with Method 2 Predictions

EPA is currently restricting the application of Method 2 for determining site-specific BAFs to nonionic organic chemicals with a log K_{ow} of 4. This restriction primarily reflects the lack of validation of this method as applied to less hydrophobic chemicals. In addition, the need for this method is greater for chemicals with higher log K_{ow}s because of the difficulties associated with detecting and measuring such chemicals in ambient water. Method 2 has not been validated for superhydrophobic (log $K_{ow} > 8$) chemicals either. Future development and evaluation of this method may lead to its application to a broader range of chemicals.

The primary assumptions and limitations for Method 1 also apply to Method 2. The primary limitation associated with Method 2 for predicting site-specific BAFs - namely, the variability of C_w^{fd} - is common to both methods. Temporal changes in C_w^{fd} are responsible for most deviations from steady state between biota, water, and sediments. The magnitude of errors associated with fluctuations in C_w^{fd} will be the same for Method 2 as for Method 1. Therefore, it may be appropriate to compare the precision of these two methods in situations where the chemical of interest *can* be measured in water. In the Monte Carlo analysis of Green Bay PCB data for Methods 1 (Appendix 3C) and 2 (Appendix 4B), BAFs determined by Method 1 were

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consistently more precise than by Method 2 when each was based on a comparable number of samples.

In deriving Equation 4-2, the assumption is made that $_{socw}$ values for reference chemicals are chosen from the same sediment data set used to calculate the BSAFs for the chemical of interest. If this cannot be done (e.g., a common data set is not available for the chemical of interest and reference chemicals) and sediment concentrations from different data sets are used instead, an error will be introduced in the Method 2 BAF prediction. This error will be proportional to any inequality in sediment concentrations between the data sets. Therefore, if the BSAF and $_{socw}$ values are not based on the same sediment data set, the investigator is cautioned to be particularly concerned with the consistency in sampling and analyses between data sets.

Although EPA recommends that C_{soc} values represent spatially averaged surface sediment contamination levels in the region affecting the organism's exposure, Method 2 should be accurate even when the C_{soc} value used for the BSAF and $_{socw}$ does not accurately represent spatially-averaged conditions. This is because the C_{soc} need only reflect the relative level of contamination of sediments over time.

Inaccuracies associated with the use of $_{socw}/K_{ow}$ from reference chemicals to estimate C_w^{fd} s for chemicals of interest under Method 2 have a linear impact on the accuracy of baseline BAFs. For example, if $_{socw}/K_{ow}$ is 10 but the estimate used is 20, the calculated baseline BAF will be greater than the true value by a factor of 2. The measurements of $_{socw}/K_{ow}$ to date indicate an expected range of 5–40 for most contamination scenarios. If the data quality considerations for choosing $_{socw}/K_{ow}$ for the chemical of interest are followed, the magnitude of the errors associated with the choice of $_{socw}/K_{ow}$ should be no greater than twofold.

The strength of Method 2 is that it utilizes measurements of relative (not absolute) differences in bioaccumulation between chemicals with structural similarity. When properly sampled, sediments provide time-stable measures of concentrations of persistent bioaccumulative chemicals in aquatic systems. Method 2 is currently the only viable method for estimating

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baseline BAFs for nonionic organic chemicals with (1) a log K_{ow} of 4, (2) concentrations in water that are often undetectable, and (3) significant rates of chemical metabolism by organisms. Important examples of chemicals with these characteristics are PCDDs, PCDFs, and non-ortho PCBs.

4.6.6 How Reliable Are Method 2 Predictions if the Sediment Organic Carbon Equilibrium Partitioning Assumption is in Error?

Equilibrium partitioning (EqP) of organic chemicals between dissolved concentrations in sediment pore water and sediment organic matter is a fundamental assumption in the Method 2 methodology for predicting BAFs. The EqP assumption is made both explicitly, in the use of $_{socw}/K_{ow}$ in equation 4-2, as well as implicitly in the use of BSAFs as tools for predicting bioaccumulation. Although equilibrium partitioning has proven to be a very powerful tool for simplifying the sorption behavior of organic chemicals in the environment, the EqP assumption has been repeatedly challenged by findings such as sorption nonlinearity (Chiou and Kile, 1998), multiphase and retarded sorption and desorption kinetics (Karickhoff and Morris, 1985), field observations of elevated partition coefficients in suspended sediment (Lohmann et al. 2005), and heterogeneous sorption properties of different classes of organic carbon (Young and Weber, 1995). Many of these factors appear to contribute relatively little variability to $_{socw}/K_{ow}$ and BSAFs, based on site-specific measurements. The justification for the use of the equilibrium partitioning assumption and the 3-phase partitioning model for organic chemicals is presented by EPA in Section 4.2.3 of TSD Volume 2 (USEPA, 2003).

Research demonstrating that specific organic chemicals (e.g., PAHs, planar PCBs) have a great affinity for particular kinds of organic matter (e.g., coal, kerogen, coke and soot, collectively known as black carbon) is a particular concern, especially given publications that specifically relate this phenomenon to variability in BSAFs. For example, Cornelissen et al. (2005) state that the observed difference in field-measured BSAFs between (planar) PAHs and (mainly nonplanar) PCBs may be explained by sorption to "carbonaceous geosorbents" or black carbon. This implies BSAF variability due to both chemical- and site- (i.e., sediment) specific factors, which are not accounted for in the Method 2 prediction methodology.

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At the present time it is difficult for EPA to evaluate the possibility that differences in the bioavailability of certain chemicals may be associated with the affinity of those chemicals for different types of organic carbon, or predict how this might affect BSAFs or Method 2 predictions. For example, the depression of BSAFs for certain planar PCB congeners is difficult to assess because of the paucity of black carbon (BC) measurements in sediments and suspended solids and the lack of measured BC-water partition coefficients. EPA has begun examining the effect of BC on chemical-specific differences in BSAFs. Burkhard et al. (2004) considered the possibility that the high sorption affinity of planar (non-ortho) PCBs for BC could explain the variability in BSAFs measured for lake trout in Lake Michigan. Kukkonen et al. (2003) has reported a BC content of 0.03% (dw) for Lake Michigan. Based upon this measurement, approximately 1% of the total organic carbon in the sediment of Lake Michigan is BC. Burkhard et al. (2004) estimate that concentrations of non-ortho PCBs (i.e., PCBs 77, 81, 126, and 169) would be lower by factors of 11x, 40x, 30x, and 235x, respectively, in pore water when BC is present as compared to when no BC is present. For the ortho substituted PCBs, concentrations in pore water would only decrease by approximately 1.5 fold. Therefore, the depression of the BSAFs for the non-ortho substituted PCBs relative to those for the ortho substituted PCBs might be attributable, in part, to reduced bioavailability. In contrast, all ortho substituted PCBs were predicted to have approximately the same reductions in bioavailability. Thus, bioavailability considerations do not appear to be the cause of the depression of BSAFs observed for only *some* of the ortho substituted PCBs in Lake Michigan. Additionally, given the relatively large differences in predicted pore water factors between the non-ortho and ortho substituted PCBs, non-ortho PCBs should have much lower BSAFs than were measured, especially PCB 169. Overall, the differences in bioavailability (measured as BSAFs) between PCB congeners could not be explained satisfactorily by the affinity of specific chemicals for BC.

The bioavailability reduction estimates made by Burkhard et al. (2004) were based on a calculation using the two phase equation of Accardi-Dey and Gschwend (2003), and BC-water distribution coefficients for ortho and non-ortho substituted PCBs (Barring et al. 2002). Burkhard et al. (2004) point out that in making their estimates of bioavailability reduction, they are extrapolating chemical concentrations in pore water by 6 to 9 orders of magnitude lower than those used for determination of the Freundlich parameters. These calculations, which imply a

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reduced bioavailability due to BC, involve extrapolations to environmental conditions which have not been tested, and are therefore highly uncertain.

Furthermore, native compounds or organic matter may compete with specific chemicals for sorption to BC (Cornelissen et al. 2005). This may attenuate or counteract the enhanced sorption of these chemicals, and limit the error made by assuming equilibrium partitioning. Further work is required to confirm the extent of enhanced sorption to BC in aquatic systems.

Other complications in the application of Method 2 may arise from enhanced partitioning of certain chemicals to BC. For example, the steady-state $_{socw}/K_{ow}$ approximated by the ratio of organic carbon contents (f_{oc} /f_{soc}) assumes that the makeup of organic carbon in the water column and sediment are similar. Gschwend and others (Gustafson et al. 1997; Accardi-Dey and Gschwend, 2002; Bushel and Gustafsson, 2000) have reported that organic carbon from anthropogenic sources, e.g., BC, have sorptive capacities different from naturally derived organic carbon. Thus, for ecosystems such as harbors and Superfund sites where a relatively large portion of the organic carbon in the sediment might arise from anthropogenic sources, the steady-state $_{socw}/K_{ow}$ may differ from the above ratio due to differences in the organic carbon composition between the water and sediments.

Additional guidance regarding the application of Method 2 may be necessary as scientific understanding of the extent and magnitude of the chemical-specific differences in partitioning behavior of different types of organic carbon improves. In the mean time, a number of precautions should be taken by the investigator to limit errors in Method 2 predictions due to this factor:

- Select reference chemicals with partitioning behavior similar to the chemical of concern. Chemicals reported to have enhanced affinity for BC include PAHs; planar (non-ortho and mono-ortho substituted) PCBs; and planar chlorobenzenes, PCDD/PCDFs and PBDEs.
- At sites where sediments contain significant amounts of BC, the three-phase model could be modified to include a fourth phase consisting of BC. Gustafsson et al. (1997) describe a methodology for estimating the partition coefficients for BC.

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- Ensure that the methods to calculate dissolved chemical fractions are used consistently throughout the application of Method 2. For example, if an adjustment to chemical bioavailability due to BC is made in sediment, a corresponding bioavailability adjustment should also be made for suspended solids in the water column.

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Appendix 4A

Modeling Simulation of BSAF Sampling Designs

Burkhard (2003) performed model simulations to understand how the variabilities in water and sediment chemical concentrations translate into the variabilities associated with BSAFs as well as BAFs based upon different sampling designs. Different models were constructed to evaluate temporal and spatial variability in chemical concentrations. As noted by Burkhard (2003), for these simulations to be meaningful the model constructs should provide reasonable representations of ecosystem conditions and chemical properties. Because the models are generic (i.e., not calibrated to site-specific data), the results are intended to compare different sampling designs in terms of the precision, and do not offer definitive predictions with known certainty. The investigator should also realize that models, including those used to perform these simulations, are being continuously updated as new data become available for testing and as scientific understanding evolves. Appendix 3D (Modeling Simulation of BAF Sampling Designs) presents the models used to evaluate temporal and spatial variability in chemical concentrations.

A number of BSAF sampling designs were evaluated by modeling chemical bioaccumulation in a river segment, assuming a mixed benthic/pelagic food web and $_{socw}/K_{ow}$ =1, for chemicals with log K_{ow}s ranging from 2 to 9. In these designs, fish and sediment were collected on the same day and sampling was repeated at fixed intervals with spacings of 1, 7, 14, 30, and 120 d (Figure 4A-1). In this figure, the uncertainty is presented as the ratio of 90th to the 10th percentile confidence limits of the BSAFs. For chemicals with log K_{ow}s greater than 5, the five sampling designs provided practically identical uncertainties for the measured BSAFs and the uncertainty was essentially independent of the number of samples collected. The collection of one set of samples (i.e., one day of sampling) provided BSAF confidence limit ratios of less than 3 for all five sampling designs. In contrast, larger uncertainties in the measured BSAFs were observed for chemicals with log K_{ow}s less than 5. For the less-hydrophobic chemicals, increasing both the number of sampling events and their spacing in time reduced the uncertainties in the measured BSAFs.

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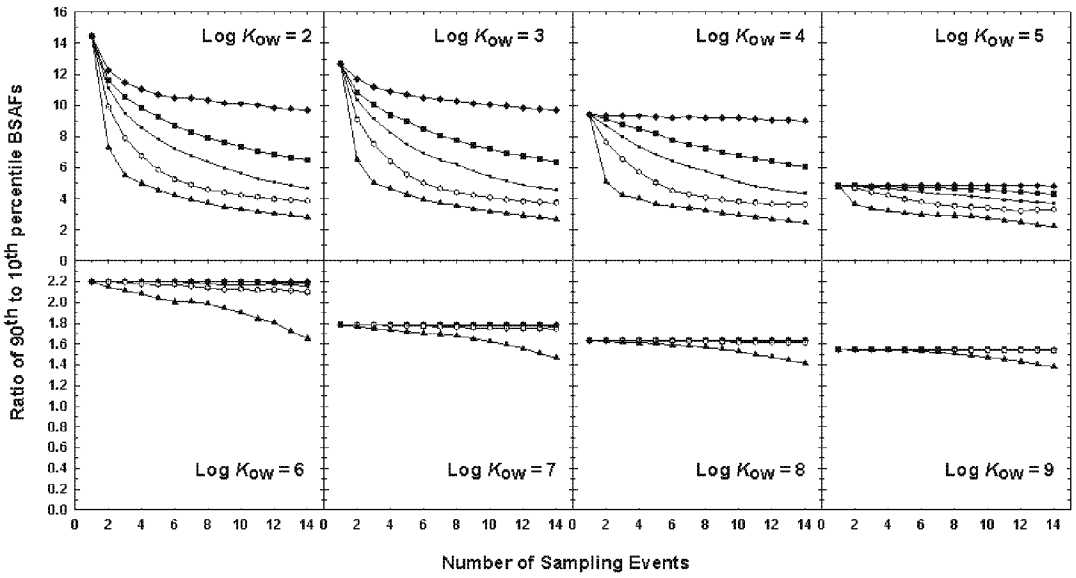

Figure 4A-1. Ratio of the 10th to 90th percentile biota–sediment accumulation factor (BSAF) for field-sampling designs consisting of concurrent fish and sediment sample collections spaced 1 ( ), 7 ( ), 14 ( ), 30 ( ), and 120 ( ) d apart, when using Mississippi River (USA) flow data for years 1955 to 1995. Results are based on modeling assumptions including (a) mixed benthic-pelagic food web and (b) $_{socw}/K_{ow} = 1$.

The simulations presented above were made assuming that the chemical was not metabolized by the fish. When metabolism does occur, the appropriate sample design for a chemical of a given K_{ow} would be best described by the sample design for a chemical with a smaller K_{ow} (with no metabolism). In this case, the *effective* reduction of K_{ow} due to metabolism would be proportional to the rate of metabolism relative to the overall depuration rate, which is the sum of elimination rates via gill and gut, the organism growth rate, and the rate of chemical metabolism.

The kinetics of chemical uptake and loss by the fish (or other aquatic organism) controls the chemical residue that resides in the organism. These kinetic processes are directly dependent upon the chemical's hydrophobicity and metabolism rate in the fish. Successful field-sampling designs should account for the chemical uptake and loss kinetics, and for the changes in chemical concentrations occurring in the fish's environment. The modeling simulations strongly

demonstrate that lower uncertainties can be obtained by using properly developed sampling design structures. The haphazard collection of samples for the measurement of chemical concentrations in biota and sediment can, and most often will, result in BSAF values with poor accuracy and large biases. Consequently, the measured values will have poor predictive power.

The modeling simulations suggest that food web structure and sediment–water chemical concentration quotients are not usually important considerations to be factored into a sampling design. Chemical concentration gradients do not add large uncertainties into the measured BSAFs beyond those caused by temporal variability alone. BSAFs can be measured with low uncertainty even when extreme spatial concentration gradients exist at the field site. However, these simulations also suggest that measurements for BSAFs probably should be designed around the more contaminated reaches of the site.

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Appendix 4B

Determining the Number of Samples to Collect for a BSAF Measurement: Monte Carlo Analysis

Monte Carlo simulation can be used to estimate how the precision of the BSAF depends upon the number of biota and sediment samples, and how the precision of the sediment-water concentration quotient ($_{socw}$) depends upon the number of sediment and water samples. The Monte Carlo method can also be used to simulate chemical concentrations in biota (for the chemical of interest and the reference chemical), sediment (chemical of interest) and water (reference chemical) simultaneously, so the investigator can determine how the precision of site-specific BAF predictions made using Method 2 depend upon the number of samples collected from each medium at the site.

The Latin Hypercube Monte Carlo generator program was used to simulate 300 chemical concentrations in biota, sediment and water. Means and variances that were calculated from log-transformed concentration data measured for PCB congeners in Green Bay (Lake Michigan) zone 3 forage and predator fish, surficial sediment and dissolved water were used as inputs to the Monte Carlo generator. Data from this zone were selected because a relatively large number of concentration measurements (93 in water, 66 in forage fish, 42 in predator fish, and 39 in sediment) were available. The sediment concentration data are presented in Appendix 4C; the data for PCB concentrations in fish and water were presented in Appendix 3B. Chemical concentrations were simulated for each of 4 congeners (PCB-18, 52, 149 and 180) in all three media (biota, sediment and water). From these simulated data, alternative numbers of biota (n_b), sediment (n_s) and water (n_w) concentrations were sampled and averaged, to simultaneously compute BSAFs, $_{socw}$s and predicted BAFs. This procedure was repeated many times, until stable distributions of BSAF, $_{socw}$ and BAF values were generated. As we will demonstrate, these distributions can be used by the investigator as estimates of the uncertainty of the BSAF, $_{socw}$, or the BAF predicted using Method 2. For example, the 90% confidence limits are estimated by the 95th and 5th percentiles of the BAF distribution. By repeating this procedure using different n_b, n_s and n_w and comparing the results, the investigator can determine a sampling

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design that meets their requirements for BSAF and/or BAF precision. Uncertainty in chemical K_{ow}s were not included in these computations, and the fugacity gradient ratio was assumed to be 1.

Monte Carlo simulations of PCB congener concentrations were made using lognormal distribution moments (mean and CV) as measured in Green Bay for dissolved water, lipid-normalized predator fish, and organic-carbon normalized surficial sediment. Additional sediment concentrations were simulated using a range of different lognormal CVs: 0.6, 0.9, 1.2, and 1.5. The variability of chemical concentrations in sediment at many sites will fall within this range of values. For example, the lognormal CVs for PCB congener concentrations in Green Bay sediment ranged from 0.71 to 1.3. The impact of different levels of variability in water concentrations was discussed in Section 3.2.1.2 and Appendix 3C.

Unless stated otherwise, concentrations in these simulations were assumed to be uncorrelated between media and between chemicals. As was the case for site-specific BAFs in Section 3.3.2, the ratios of 90% confidence limits (upper CL/lower CL) were used as measures of the uncertainty of the distributions of BSAFs, $_{socw}$s and BAFs in each simulation.

How is the Uncertainty of BSAF and $_{socw}$ Affected by the Number of Sediment Samples and Different Chemical Concentration Variances?

The uncertainty of BSAFs and $_{socw}$ calculated in the Monte Carlo simulations were sensitive to the number of sediment samples, and this sensitivity increased with the variability of the sediment chemical concentrations. For example, Figure 4B-1 shows how the 90% confidence limit ratio (CLR) for the BSAF varies based upon (1) the number of samples used to calculate the mean sediment concentration, and (2) the coefficient of variation (CV) of the underlying population of chemical concentrations. The BSAF CLRs in Figure 4B-1 are averages for the four PCB congeners, and the BSAFs were calculated using mean chemical concentrations calculated from 6 biota samples. These results are also shown in Table 4B-1, which includes CLRs for BSAFs calculated using mean chemical concentrations from different numbers of sediment and fish samples. For highly variable chemical concentrations in sediment, increasing the number of sediment samples used to calculate the mean concentration has a significant impact on reducing

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the uncertainty of BSAFs, up to a sample size of about 6. Collecting additional sediment samples (i.e., greater than 10) has little effect on the precision of BSAFs, as illustrated by the CLRs in Figure 4B-1.

Figure 4B-2 is a similar plot of the 90% confidence limit ratio (CLR) for $_{socw}$. The CLRs in Figure 4B-2 are averages for the four PCB congeners, and were again calculated using mean chemical concentrations calculated from 6 water samples. These results are also shown in Table 4B-2, which includes CLRs for $_{socw}$ calculated using mean chemical concentrations from different numbers of sediment and water samples. As was the case for the BSAF, increasing the number of samples used to calculate the mean sediment concentration has a significant impact on reducing the uncertainty of $_{socw}$, up to a sample size of 6. Collecting additional sediment samples (i.e., greater than 10) has little effect on the uncertainty of $_{socw}$ s.

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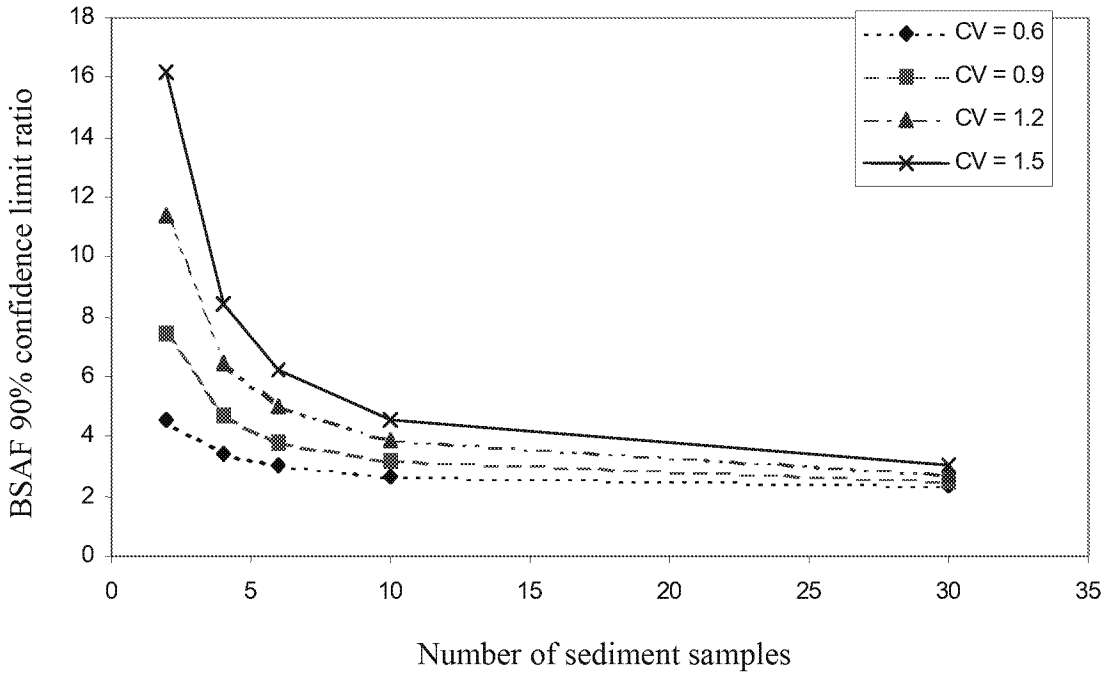

Figure 4B-1. 90% confidence interval ratio for BSAF as function of number of sediment samples (Average results for 4 PCB congeners are plotted; BSAFs were calculated using 6 biota samples drawn from Green Bay predator fish data).

Table 4B-1. 90% Confidence Interval Ratios for BSAF as Function of the Variability in Chemical Concentrations in Sediment

A. Chemical Concentrations Measured in 2 Fish Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	6.43	9.62	14.2	19.5
4	5.12	6.53	8.68	10.8
6	4.68	5.60	6.98	8.34
10	4.34	4.88	5.68	6.48
30	4.00	4.17	4.43	4.71

B. Chemical Concentrations Measured in 4 Fish Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	5.05	7.99	12.0	16.7
4	3.82	5.11	7.00	8.91
6	3.42	4.26	5.49	6.74
10	3.10	3.59	4.32	5.06
30	2.77	2.93	3.18	3.42

C. Chemical Concentrations Measured in 6 Fish Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	4.59	7.44	11.4	16.1
4	3.40	4.68	6.47	8.43
6	3.01	3.83	5.00	6.26
10	2.69	3.17	3.87	4.58
30	2.36	2.52	2.77	3.01

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Table 4B-1 (Continued). 90% Confidence Interval Ratios for BSAF as Function of the Variability in Chemical Concentrations in Sediment

D. Chemical Concentrations Measured in 10 Fish Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	4.24	7.02	10.8	15.3
4	3.06	4.34	6.04	7.97
6	2.67	3.50	4.63	5.84
10	2.35	2.84	3.53	4.20
30	2.02	2.19	2.43	2.67

E. Chemical Concentrations Measured in 30 Fish Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	3.89	6.59	10.3	15.0
4	2.72	4.01	5.65	7.47
6	2.33	3.19	4.27	5.48
10	2.00	2.53	3.18	3.87
30	1.64	1.83	2.09	2.33

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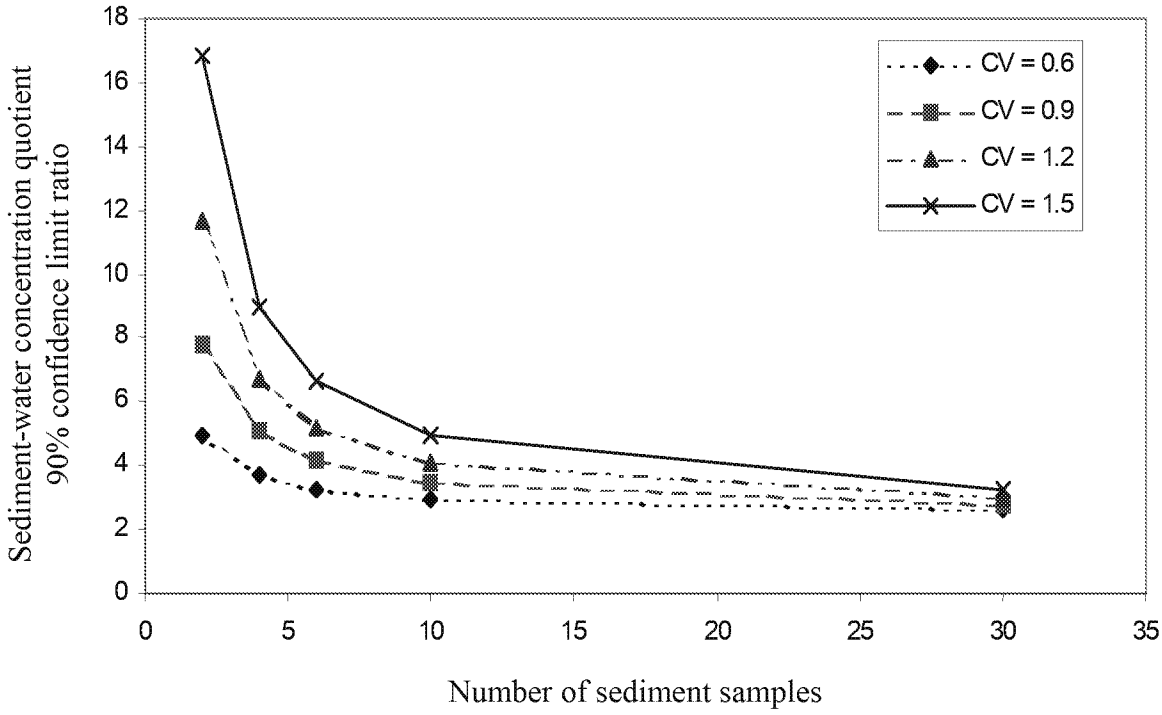

Figure 4B-2. 90% confidence interval ratio for $_{socw}$ as function of number of sediment samples. (Average results for 4 PCB congeners are plotted; $_{socw}$s were calculated using 6 water samples drawn from Green Bay data).

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Table 4B-2. 90% Confidence Interval Ratios for $_{Socw}$ as Function of the Variability in Chemical Concentrations in Sediment

A. Chemical Concentrations Measured in 2 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	7.46	11.2	15.7	21.9
4	6.04	7.76	9.70	12.2
6	5.55	6.68	7.89	9.51
10	5.18	5.86	6.52	7.49
30	4.82	5.04	5.24	5.56

B. Chemical Concentrations Measured in 4 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	5.52	8.58	12.6	17.9
4	4.25	5.72	7.39	9.71
6	3.83	4.79	5.85	7.27
10	3.50	4.07	4.70	5.53
30	3.18	3.37	3.56	3.85

C. Chemical Concentrations Measured in 6 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	4.91	7.81	11.7	16.8
4	3.69	5.06	6.71	8.99
6	3.28	4.19	5.21	6.67
10	2.96	3.51	4.10	4.92
30	2.63	2.82	3.02	3.28

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Table 4B-2 (continued). 90% Confidence Interval Ratios for $_{Socw}$ as Function of the Variability in Chemical Concentrations in Sediment

D. **Chemical Concentrations Measured in 10 Water Samples**

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	4.43	7.23	11.0	15.8
4	3.23	4.56	6.17	8.25
6	2.84	3.72	4.73	6.10
10	2.52	3.06	3.63	4.42
30	2.19	2.38	2.58	2.86

E. **Chemical Concentrations Measured in 30 Water Samples**

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	3.95	6.66	10.3	15.1
4	2.78	4.08	5.69	7.60
6	2.39	3.26	4.30	5.52
10	2.06	2.60	3.21	3.95
30	1.71	1.92	2.12	2.41

What are the Confidence Interval Ratios for Method 2 BAFs?

Because concentrations in biota, sediment and water were simulated for 4 chemicals simultaneously, it was possible to calculate 3 BAFs for each chemical using Method 2 (one chemical as the "chemical of interest" and 3 as "reference chemicals"). CLRs for Method 2 BAF predictions for PCB-180 are shown as a function of sediment sample numbers in Figure 4B-3. In this figure, the CLRs are plotted separately for BAF predictions made using the different reference chemicals. Although the trend of declining CLRs (and lower uncertainty) as a function of the number of sediment samples is consistent for BAFs calculated using different reference chemicals, some differences are also apparent. For example, sediment concentrations for PCB-52 are significantly more variable (CV=1.29) than for the other chemicals, and this is reflected in

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higher CLRs for Method 2 BAF predictions when this congener is used as the reference chemical.

The CLRs for Method 2 BAF predictions were found to be fairly consistent for different chemicals of interest. This is shown in Figure 4B-4, which plots CLRs for Method 2 BAFs for each congener as the chemical of interest, again as a function of sediment sample size. The CLRs in this figure were averaged across BAFs calculated using the three reference chemicals.

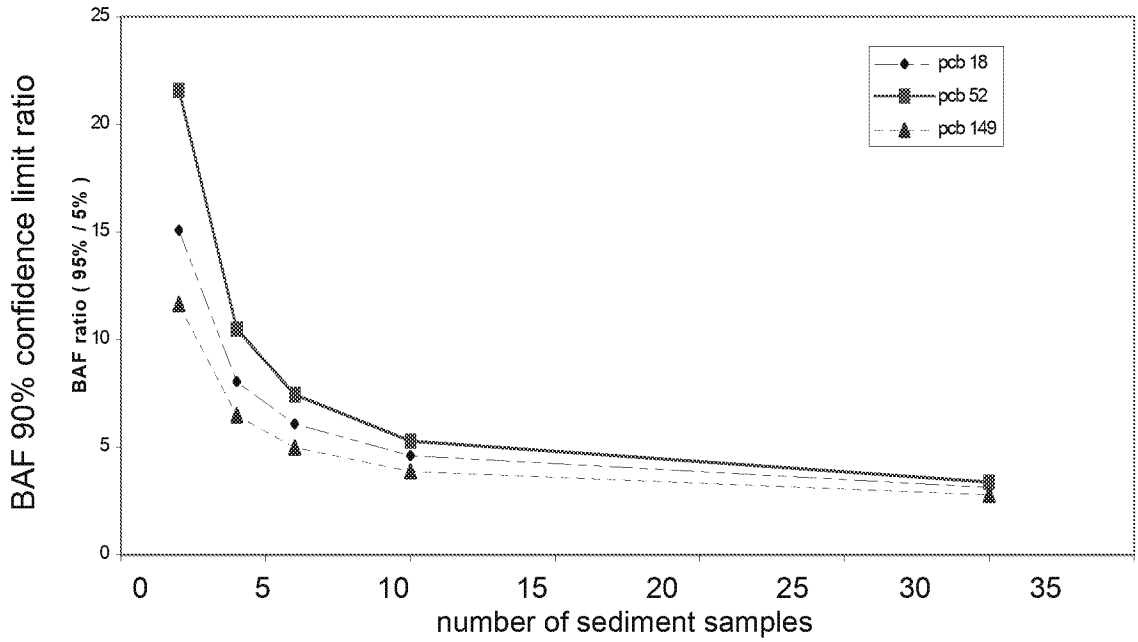

Figure 4B-3. 90% confidence interval ratio for PCB-180 Method 2 BAFs as a function of the number of sediment samples (results shown for 10 biota and 10 water samples). In each curve plotted in this figure, the BAF confidence interval ratios were calculated by Method 2 using a different PCB congener as a reference chemical.

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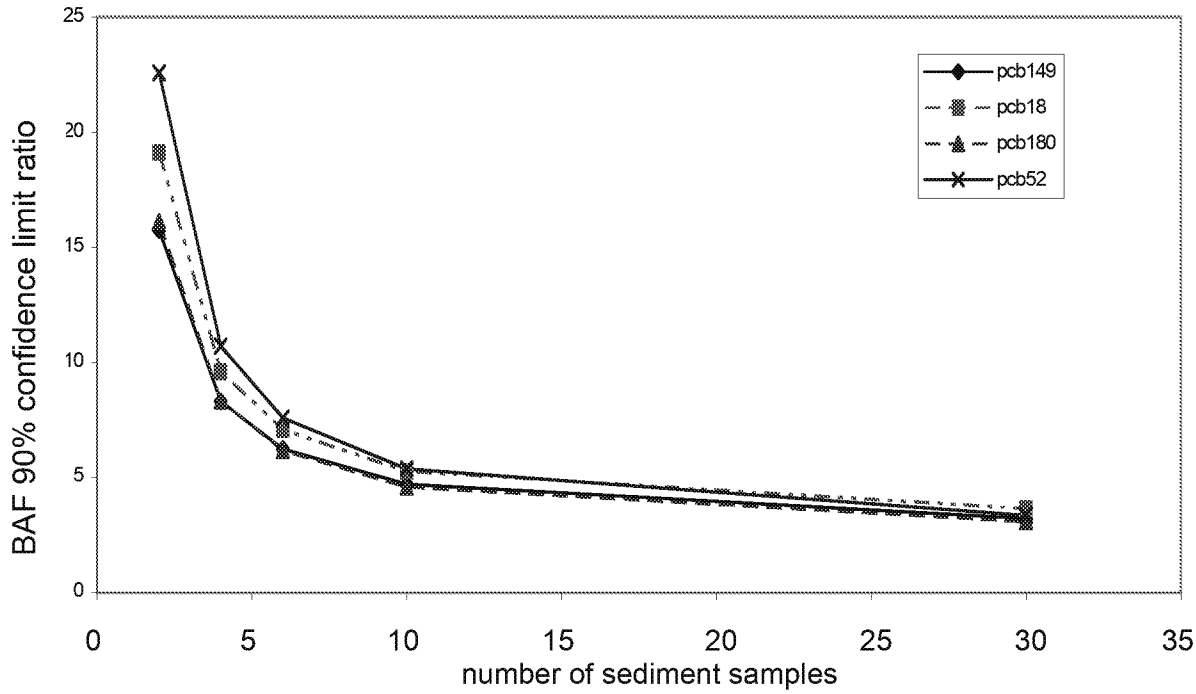

Figure 4B-4. 90% confidence interval ratio for four PCB congener Method 2 BAFs as a function of the number of sediment samples (results shown for 10 biota and 10 water samples). In this Figure, BAF confidence interval ratios are plotted as separate curves for each PCB congener.

How Does the Variability of Chemical Concentrations in Sediment Affect the Precision of Method 2 BAF Predictions?

Additional Monte Carlo simulations were run and analyzed, in order to develop generalized guidance for selecting sample sizes that would be applicable for different sites. To do so, the simulations were repeated using different CVs for chemical concentrations in sediment. As discussed above, the variability of chemical concentrations in sediment affected the uncertainty of BSAFs and $_{socw}$, particularly for small sediment sample sizes (n_s 6). For Method 2 BAFs, the results are similar to those for BSAF and $_{socw}$, although the CLRs are much larger. This is shown in Figure 4B-5, which plots the Method 2 BAFs predicted for PCB-149 as a function of the chemical concentration variance in sediment and the number of sediment samples used to calculate the BSAF, $_{socw}$ and BAF. The results shown in Figure 4B-5 are for Method 2 BAFs calculated with mean chemical concentrations based on 10 biota and 10 water samples. The results for PCB-149 are also presented in Table 4B-3, along with CLRs for other combinations of biota, sediment and water sample sizes. Each sub-table presents the 90% BAF CLR as a function of the number of sediment samples and the underlying variability of chemical concentrations in sediment, for a specific number of fish and water concentrations. For example, Table 4B-3.a is a tabulation of results for 2 fish and 2 water samples. For this case, if the BAF is predicted using 2 sediment samples, the 90% BAF CLRs vary from 16 to 75 depending upon the variability of chemical concentrations in the sediment. If 6 sediment samples are used to make the Method 2 prediction, the resulting 90% BAF CLRs are much lower, varying from 10 to 23. If 30 sediment samples are used, the 90% BAF CLRs are further reduced to 8 to 10. The other sub-tables in Table 4B-3 present 90% BAF CLRs for 4 fish and water samples (Table 4B-3.b), 6 fish and water samples (Table 4B-3.c), 10 fish and water samples (Table 4B-3.d), and 30 fish and water samples (Table 4B-3.e).

As was the case for BSAFs and $_{socw}$s, the results in Table 4B-3 demonstrate that only small reductions in the uncertainty of Method 2 BAF predictions are gained using sediment sample sizes larger than about 6. Once the number of samples exceeds about 6, the reductions in BAF prediction CLRs become incrementally much smaller. This is the case even when the variability of chemical concentrations in sediment is large. Depending upon the requirements for

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predictive BAF uncertainty, exceeding sample sizes of 10 appears to be warranted only for sites having very high variability in chemical concentrations in sediment.

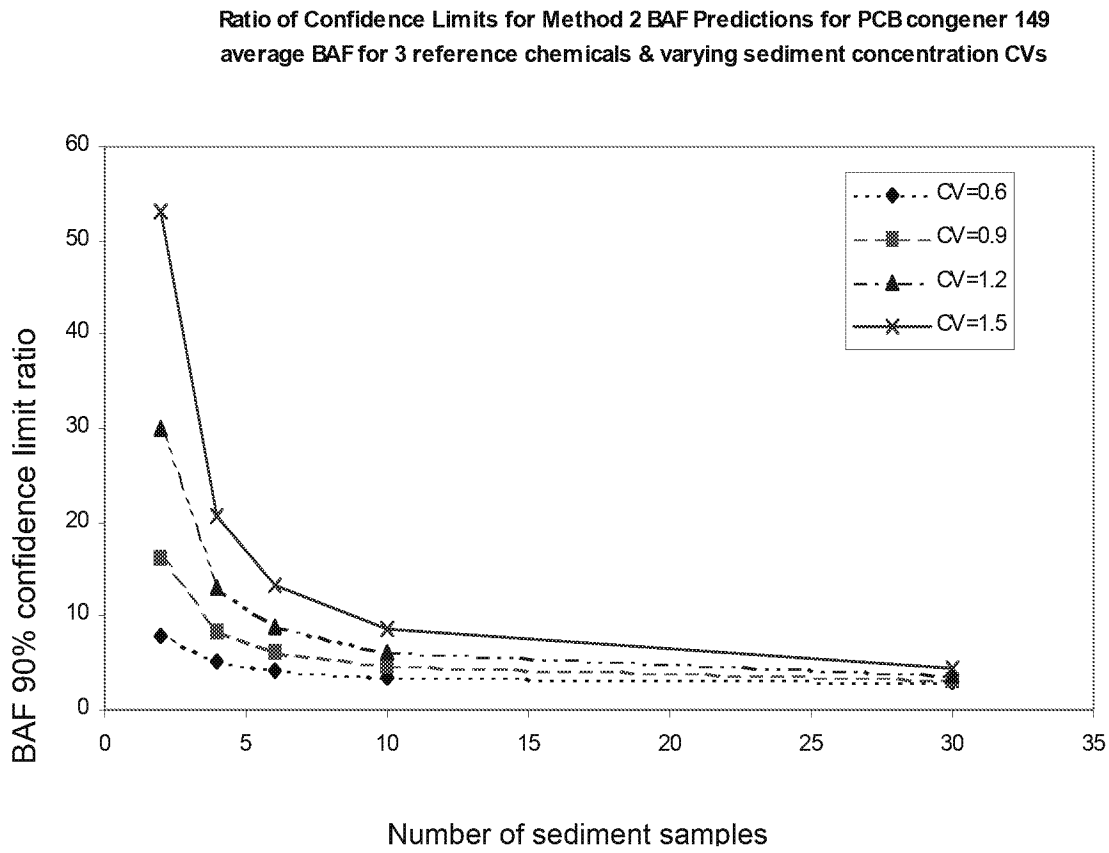

Figure 4B-5. 90% confidence interval ratio for PCB-149 Method 2 BAFs as a function of the number of sediment samples and variability of chemical concentrations in sediment (results shown for 10 biota and 10 water samples). In each curve plotted in this figure, the BAF confidence interval ratios were calculated by using a different coefficient of variation (CV) for sediment concentrations.

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Table 4B-3. 90% Confidence Interval Ratios for Method 2 BAF Predictions for PCB Congener 149 as a Function of the Variability in Chemical Concentrations in Sediment

A. Chemical Concentrations Measured in 2 Fish and 2 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	15.5	27.6	47.2	75.3
4	11.3	16.0	22.9	33.7
6	9.96	12.8	16.9	23.2
10	8.96	10.5	12.7	16.1
30	8.01	8.36	9.11	10.0

B. Chemical Concentrations Measured in 4 Fish and 4 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	10.5	20.0	35.9	60.9
4	7.18	11.0	16.4	25.4
6	6.17	8.47	11.7	16.8
10	5.41	6.64	8.4	11.1
30	4.65	4.96	5.55	6.29

C. Chemical Concentrations Measured in 6 Fish and 6 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	9.05	17.9	32.6	56.3
4	5.98	9.46	14.6	22.8
6	5.06	7.2	10.1	14.9
10	4.34	5.5	7.1	9.61
30	3.64	3.96	4.48	5.18

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Table 4B-3 (continued). 90% Confidence Interval Ratios for Method 2 BAF Predictions for PCB Congener 149 as a Function of the Variability in Chemical Concentrations in Sediment

D. Chemical Concentrations Measured in 10 Fish and 10 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	7.95	16.2	30.0	53.1
4	5.07	8.33	13.1	20.7
6	4.2	6.2	8.97	13.3
10	3.53	4.65	6.12	8.49
30	2.87	3.19	3.67	4.33

E. Chemical Concentrations Measured in 30 Fish and 30 Water Samples

Number Of Sediment Samples	CV=0.6	CV=0.9	CV=1.2	CV=1.5
2	6.87	14.7	27.6	49.6
4	4.2	7.25	11.7	18.8
6	3.39	5.25	7.87	11.8
10	2.74	3.82	5.22	7.29
30	2.08	2.44	2.87	3.52

What are the Effects of Chemical Concentration Correlations on Method 2 BAF Predictions?

The BAFs predicted above using Method 2 were based upon simulated chemical concentrations in biota, sediment and water that were uncorrelated and independent. In a real ecosystem, however, the concentrations of bioaccumulative chemicals are likely be correlated in biota, sediment and water. Two different kinds of correlation are possible: *within-chemical* correlation of the concentrations of a specific chemical between different sampled media, and *within-media* correlation between different chemicals. Within-chemical correlation is generally expected, due to factors such as the magnitude of chemical loading; in contrast, we expect the transport, partitioning and bioaccumulation processes to differ between chemicals due to their

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physicochemical properties. Within-media correlation of chemical concentrations is also expected and could result, for example, if the concentrations of multiple chemicals were higher in one or a subset of sediment samples. Since Method 2 BAF predictions are based on concentrations of at least 2 chemicals (chemical of interest and one or more reference chemicals) in all three media, either kind of correlation may affect the uncertainty of the BAF prediction.

The Monte Carlo simulations of chemical concentrations were repeated to evaluate how within-chemical and within-media correlation would alter the estimates of BAF uncertainty presented in Section 4.4.5. To simulate within-chemical correlation, rank correlation coefficients of 0.5 were specified for each PCB congener between fish, sediment and water concentrations. The resulting BAF CLRs are shown as a function of sediment sample size in Figure 4B-6, along with the uncorrelated simulation results for PCB-52. Results were similar for the other congeners (not shown). Within-chemical correlations were found to be mildly helpful in terms of reducing the uncertainty of Method 2 BAFs; on average, the 0.5 correlation reduced the CLRs by 21%.

The same approach was used to simulate within-media correlation of chemical concentrations. Rank correlation coefficients of 0.5 were specified in each sample medium (biota, sediment and water) between PCB congener concentrations. The resulting BAF CLRs are again shown as a function of sediment sample size in Figure 4B-6. Within-media correlation, especially the correlation between chemical concentrations in sediment, significantly reduced the uncertainty of Method 2 BAF predictions when few sediment samples are collected. When only two sediment samples are used to calculate the BSAF and $_{socw}$, the 0.5 correlation reduced the CLR by 50% in comparison to the uncorrelated simulation. For 4 sediment samples, the reduction in the CLR was 40%, and for 6 samples the CLR reduction was 30%. Overall, concentration correlations were found to be helpful in terms of improving the precision of Method 2 BAF predictions; this was especially the case when relatively few samples were drawn from sediment concentrations that were correlated between chemicals. Such correlations are reasonable to expect in sediment data from a specific site, and help to explain why Method 2 BAF predictions are so robust. In many cases, the investigator will not know *a priori* whether chemical correlations exist; these simulations illustrate that a conservative number of samples will be specified if chemical concentrations are assumed to be uncorrelated. In other words, the

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investigator who determines the number of samples assuming uncorrelated chemical concentrations (i.e., the sample size guidance in Tables 4B-1 through 4B-3) can expect to determine a BAF *no more* uncertain than indicated in these tabulations.

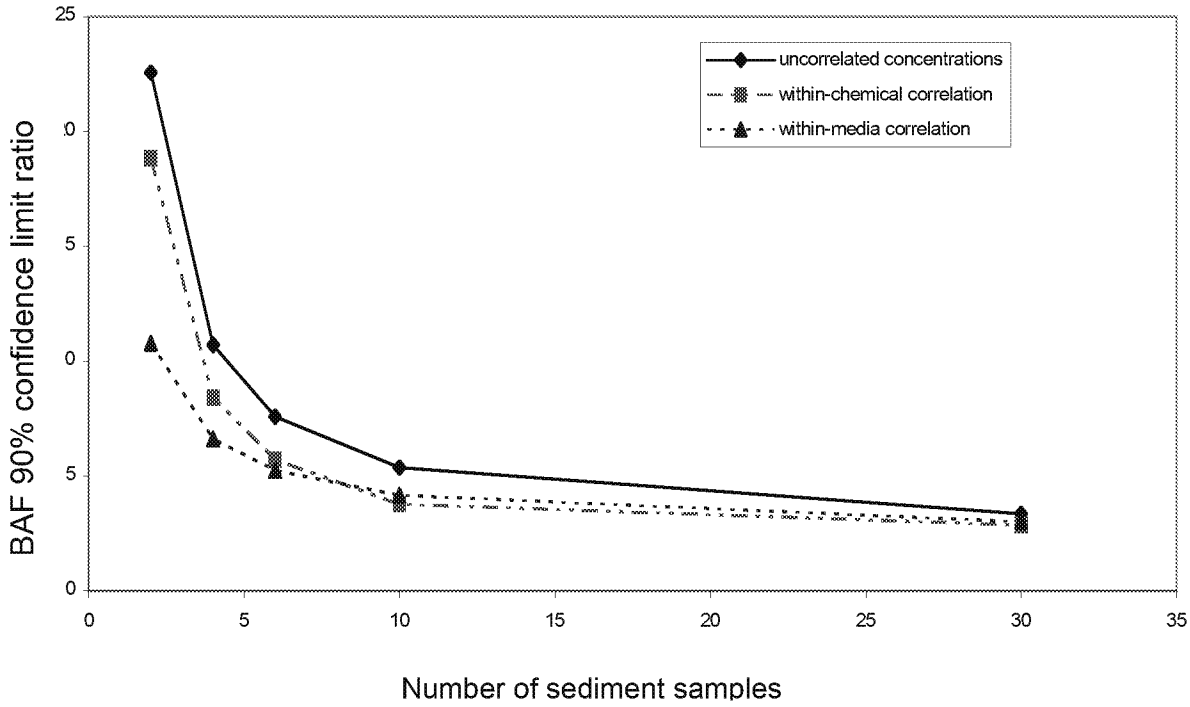

Figure 4B-6. 90% confidence interval ratio for PCB-52 Method 2 BAFs as a function of concentration correlations (results shown for 10 biota and 10 water samples). In each curve plotted in this figure, the BAF confidence interval ratios were calculated from simulated fish, sediment and water concentrations incorporating different assumptions regarding correlations between the data.

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Appendix 4C
Green Bay Mass Balance PCB Congener Concentrations: Organic Carbon-Normalized Surficial (0-1 cm) Sediment

year	zone	sediment station	percent organic carbon	PCB 18 (ng/g-SOC)	PCB 52 (ng/g-SOC)	PCB 149 (ng/g-SOC)	PCB 180 (ng/g-SOC)
1989	GB0Z3B	58	5.09	41	61	21	12
1989	GB0Z3A	54	5.82	33	48	20	14
1990	GB0Z3B	52	4.78	37	54	18	14
1988	GB0Z3B	48	9.53	40	59	25	28
1987	GB0Z3B	47	NA				
1988	GB0Z3B	44	7.76	22	27	8.6	11
1988	GB0Z3B	43	9.28	76	117	45	47
1988	GB0Z3B	43	9.28	113	147	51	31*
1988	GB0Z3B	42	8.49	61	108	41	43
1989	GB0Z3B	40	3.83	40	57	18	14
1988	GB0Z3B	39	9.03	110	155	50	31
1987	GB0Z3B	39	9.03	132	155	47	49
1990	GB0Z3B	38	8.27	75	134	34	6.2
1988	GB0Z3A	33	8.23	66	115	43	17
1988	GB0Z3B	32	8.08	69	90	31	17
1987	GB0Z3B	32A	7.39	55	105	29	9.1
1987	GB0Z3B	32A	7.39	134	229	31	15*
1987	GB0Z3B	31	7.41	198	284	86	72
1987	GB0Z3B	30	7.89	169	223	62	7.6
1989	GB0Z3A	27	7.95	171	223	71	38
1987	GB0Z3A	27A	6.99	150	275	67	33
1989	GB0Z3B	26	8.01	228	6.4*	107	32
1987	GB0Z3B	24	6.39	240	289	67	31
1987	GB0Z3A	22	6.52	238	367	78	26*
1987	GB0Z3A	22	6.52	173	336	55	87
1987	GB0Z3A	22	6.52	304	414	134	58*
1987	GB0Z3B	21	7.82	237	314	83	77
1988	GB0Z3B	20	6.83	296	339	71	97
1988	GB0Z3B	20	6.83	243	199	38	19
1988	GB0Z3A	18	7.51	85	141	40	38
1989	GB0Z3A	17	7.54	139	207	49	38
1987	GB0Z3A	17	7.54	271	357	84	72
1988	GB0Z3B	16	6.91	416	628	123	97
1989	GB0Z3A	13	4.12	177	183	38	28
1987	GB0Z3B	12	5.56	545	755	143	139
1989	GB0Z3A	11	0.17	155	28*	94	73*
1988	GB0Z3A	10	1.51	262	344	80	69
1987	GB0Z3A	10A	3.84	326	381	84	109
1988	GB0Z3B	9	4.43	315	483	95	104
1988	GB0Z3B	8	5.45	505	702	126	104

Note: * Denotes sediment PCB concentration below limit of quantification (LOQ); replacement value estimated using the Maximum Likelihood method of El-Shaarawi and Dolan (1989).

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5. ESTIMATING SITE-SPECIFIC BAFs BY EXTRAPOLATION, PREDICTION OR RECALCULATION

The previous 2 sections of this TSD have described EPA's preferred methods of determining site specific BAFs: determining a BAF directly based on site-specific measurements, or determining a BSAF based on site-specific measurements and then predicting a BAF from the BSAF. Another approach to determine site-specific BAFs is to estimate a site-specific BAF indirectly using one of the other methods described in TSD Volume 2 (USEPA, 2003). These methods include extrapolating site-specific BAFs from BSAFs, predicting BAFs using laboratory-measured bioconcentration factors (BCFs) or octanol-water partition coefficients (K_{ow}s) coupled with food chain multipliers, or recalculating site-specific BAFs from baseline BAFs. EPA expects that variations of these methods, as described in this section, may be used to derive site-specific BAFs.

ALTERNATIVES FOR ESTIMATING SITE-SPECIFIC BAFs:

- Extrapolating site-specific BAFs from BSAFs measured at another site (Method 3, with 2 options)
  - 3a. BSAF extrapolation
  - 3b. BEF extrapolation
- Predicting BAFs using a BCF coupled with food chain multipliers (Method 4, with 2 options):
  - 4a. Laboratory-measured BCFs
  - 4b. BCFs estimated using K_{ow}s
- Recalculating site-specific BAFs from baseline BAFs (Method 5, with 2 options:
  - 5a. Adjustment for site-specific lipid content, and/or
  - 5b. Adjustment for site-specific DOC

EPA considers the BAF estimation methods described in this section to be less preferred than the direct determination of the BAF or BSAF based on measurements made at the site, because these estimation methods may not capture all of the site-, chemical- and/or species-

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specific factors that influence chemical bioaccumulation. In addition, the estimation methods rely upon assumptions that may be difficult to confirm. As a result, the site specific BAFs estimated by these methods are uncertain due to factors beyond those discussed in previous sections (i.e., sampling bias, measurement errors, etc.). This does not mean that these estimation methods cannot produce a good site specific BAF. Rather, it is meant to caution the investigator to carefully consider whether a particular BAF estimation method is appropriate given the characteristics of the chemical, organism and site.

The investigator should also consider whether the application of these methods to estimate site-specific BAFs will improve upon the accuracy of the national BAFs for a particular site. As discussed in Section 2, EPA believes that national BAFs are broadly applicable to sites throughout the United States and achieve an acceptable degree of accuracy; because national BAFs are derived using a methodology intended to produce national average values for BAFs at each trophic level. EPA also recognizes that conditions, parameters, etc. at a site could be different from the representative values used in the National methodology calculations. In the national methodology, default values were used for many important ecosystem and food web parameters. These include POC and DOC concentrations, trophic-level specific lipid contents, trophic structures, $_{socw}/K_{ow}$, and other parameters (USEPA, 2003). The investigator should view the derivation of site-specific BAFs as a process to improve upon the accuracy of the national BAFs for a particular site. EPA expects that in most instances, the derivation of site-specific BAFs will be motivated by some knowledge or expectation that unique site-specific factors may cause BAFs to diverge from the national values. These factors include (for example): fish consumption patterns that are substantially different than national averages; species of aquatic organisms that have not been previously sampled or for which trophic level or feeding preference is unknown; and sediment-water chemical distribution, tissue lipid content, POC and/or DOC concentration significantly different than the values assumed in the national methodology. In cases such as these, the derivation of site-specific BAFs would likely improve the accuracy of bioaccumulation estimates and, ultimately, the AWQC for the chemical of concern at that site.

The three alternatives for estimating site specific BAFs are presented and discussed in the following sections. Section 5.1 addresses Methods 3a and 3b, estimating site-specific BAFs by

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extrapolating BSAFs or BEFs. Predicting site-specific BAFs using BCFs and food chain multipliers (Methods 4a and 4b) is covered in Section 5.2. Method 5, recalculating site-specific BAFs from baseline or national BAFs, is addressed in Section 5.3.

5.1 ESTIMATING SITE-SPECIFIC BAFs BY EXTRAPOLATING BSAFs OR BEFs (METHODS 3A AND 3B)

One alternative for estimating site specific BAFs is based upon extrapolating BSAFs from a reference site to the site of interest. The investigator may extrapolate trophic level-specific BSAFs measured at a reference site using one of two approaches. The first approach is to directly extrapolate a high-quality BSAF to the site, when one has been determined for the chemical of interest at another site. Alternatively, if a high-quality BSAF for a reference chemical is available for the site, and BSAFs for that reference chemical and the chemical of interest are available at another site, then the investigator can use a bioaccumulation equivalence factor (BEF, defined as the ratio between BSAFs for the chemical of interest and the reference chemical) to extrapolate a BSAF. Since these are actually two related methods, we refer to BSAF extrapolation as Method 3a and extrapolation of a BEF as Method 3b. Figure 5-1 presents a decision framework flowchart for selecting an applicable BAF derivation method, based upon the BSAFs that may be available to the investigator. For either method, conversion of the BSAF into a site-specific baseline BAF is accomplished using Method 2 of EPA's bioaccumulation methodology. Methods 3a and 3b are appropriate for moderate to highly hydrophobic nonionic organic chemicals, and to certain ionic organic chemicals for which similar lipid and organic carbon partitioning behavior applies. Methods 3a and 3b have the greatest potential for highly hydrophobic nonionized organic chemicals that may be metabolized in the food chain and are difficult and/or expensive to measure as freely dissolved concentrations in water. Since a BSAF is based on lipid and organic carbon normalized chemical concentrations (Equation 4-1), no other adjustment for these factors is necessary.

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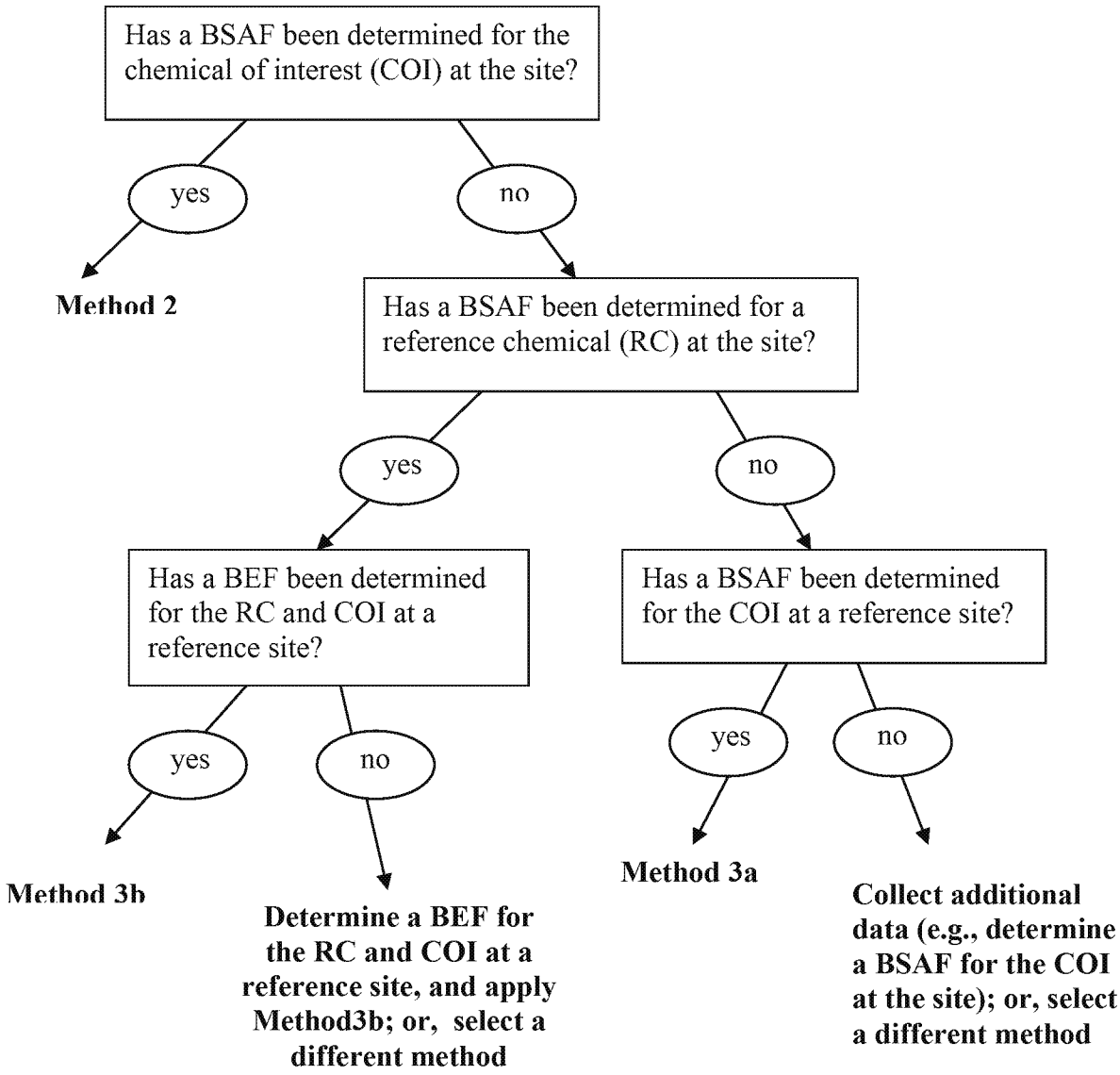
Site-Specific BAF Method 3

- Extrapolating site-specific BAFs from BSAFs measured at another site, with 2 options:
  - 3a. BSAF extrapolation
  - 3b. BEF extrapolation

Figure 5-1. Decision framework for selecting a site-specific BAF derivation method based on BSAFs



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BSAFs have been used by EPA for predicting chemical residues in aquatic organisms from contaminated sediments, especially for Superfund sites (USEPA, 1999). A site-specific BSAF (i.e., a BSAF determined at the site) is clearly most desirable when making predictions, because this BSAF incorporates all processes and conditions influencing bioaccumulation at the site. When a BSAF is determined by measurements at the site, Method 2 is used to predict the site-specific BAF, as described in Section 4. However, BSAFs are unavailable for many sites, and high-quality BSAFs determined from measurements in other ecosystems may be used in developing the site-specific BAF for the site of interest. This is Method 3a of the site-specific BAF methodology: direct extrapolation of a BSAF determined for the chemical of interest at a reference site to a site of interest. BSAF extrapolation has received increasing attention as a method for predicting site-specific BAFs (Wong et al. 2001), as BSAFs become more widely available. As discussed in Section 4, BSAFs are useful measures of bioaccumulation for organic chemicals because: (1) they do not require difficult (and potentially highly variable) measurements of chemical concentrations in water, (2) they remove the site-specific variability in BAFs due to differences in the sediment-water concentration quotient, $_{socw}$, and (3) they remove the site-specific variability due to differences in organic carbon and lipid contents.

BSAFs can be adjusted, using ratios predicted by food chain bioaccumulation models, to account for differences between sites in the degree of connection of fish to benthic/pelagic food chains, as well as differences between sites in the sediment-water disequilibrium due to differences in chemical loading histories (Burkhard et al., 2006). This approach, called hybrid bioaccumulation modeling, uses mechanistic bioaccumulation models to assist in extrapolating field-measured BSAFs by explicitly accounting for the differences between ecosystems. Although additional work is required to fully evaluate and develop the hybrid extrapolation approach for routine application, it appears promising as a method for improving the accuracy of BSAF extrapolation.

When BSAFs from one ecosystem are directly applied to another ecosystem (e.g., Method 3a), the investigator is assuming that the underlying conditions and parameters affecting bioaccumulation are the same between the site of interest and the reference site where the BSAFs were determined. This implicit assumption is often not appreciated by users of BSAF data. As

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discussed by Burkhard et al. (2005), the major conditions and parameters incorporated into a measured BSAF are:

1. the distribution of the chemical between the sediment and water column,
2. the relationship of the food chain/web to water and sediment,
3. the length of the food web (or trophic level of the organism – although this is normally accommodated in the trophic-level specific BAF calculation),
4. bioavailability of the chemical due to amounts and types of organic carbon in the ecosystem (although bioavailability differences between sites are largely accommodated by expressing BSAFs and BAFs in terms of concentrations normalized to organic carbon, lipid, and freely dissolved fraction in water), and
5. metabolic transformation rates of the chemical within the food web.

The first four factors can vary widely among ecosystems. In contrast, the fifth factor will, in all likelihood, vary much less among ecosystems. Significant unexplained variability can also arise from sampling and analytical factors. This unfortunately complicates, to an unknown degree, examples provided later in this section to demonstrate the methods with actual data.

The validity of BSAF extrapolation can be directly evaluated by comparing BSAFs determined at sites that differ in terms of the conditions, parameters, and connections that affect chemical bioaccumulation. Wong et al. (2001) measured BSAFs for *p,p'*-DDE in white suckers that ranged from 1.7 to 27 (with a median value of 8.8) across 36 different riverine ecosystems. These authors concluded that BSAF extrapolation was a useful tool for estimating bioaccumulation in rivers, but cautioned that variability in BSAF values between sites (and between different kinds of sites, e.g. rivers and lakes) might limit the accuracy and utility of this approach. Burkhard et al. (2003b) measured very similar BSAFs for 93 PCB congeners in 6 fish species across 6 spatial zones in Green Bay, Lake Michigan (average BSAF = 7.8; average congener-specific minimum and maximum values ranging from 1.3 to 25), and for 125 PCB congeners in 6 fish species at 6 locations in the Hudson River (average BSAF = 7.7; average congener-specific minimum and maximum values ranging from 2.5 to 11). Average congener-specific BSAFs determined in Green Bay and the Hudson River are reported in Appendix 5C. In

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Green Bay, the variability in BSAFs between spatial zones for a particular congener and fish species was found to be comparable to the variability of baseline BAFs.

Sets of BSAFs across ecosystems have consistent, if not identical, scaling, ranking, or ordering of the individual chemicals (Burkhard et al., 2005). When BSAF values are plotted for one ecosystem against another, chlorinated pesticides (Figure 5-2), PCDD/Fs (Figure 5-3), PCBs (Figure 5-4), and PCBs together with PCDD/Fs (Figure 5-5) fall on a line with slopes close to 1.0 and have Spearman's coefficient of rank correlation that are also close to 1.0.

The highly significant relative ranking phenomenon appears to occur in ecosystems despite their differences, or errors or biases in the measurements used to determine the BSAFs. This behavior holds for chemicals metabolized by fish, i.e., PCDD/Fs (Kleeman et al. 1988; Opperhuizen et al. 1990), as well as for chemicals with substantially lower rates of metabolism in fish, i.e., PCBs. The demonstration of consistent scaling/ranking of individual BSAFs across ecosystems is quite remarkable. It creates opportunities for improving our understanding of bioaccumulation processes in aquatic ecosystems, and for improving the accuracy of site-specific bioaccumulation factors that are estimated by extrapolating BSAFs (Burkhard et al. 2005). In particular, extrapolating a ratio of BSAFs (a bioaccumulation equivalency factor or BEF) from another ecosystem and adjusting it using a BSAF for a reference chemical at the site, improves the Method 3 extrapolation by incorporating the BSAF ranking behavior in the methodology. This is the basis for Method 3b. By incorporating more information from other ecosystems (e.g., a BEF instead of a BSAF) and adjusting this information to reflect conditions at the site (via measuring BSAFs for reference chemicals), an improved estimate for the site-specific BAF can be obtained.

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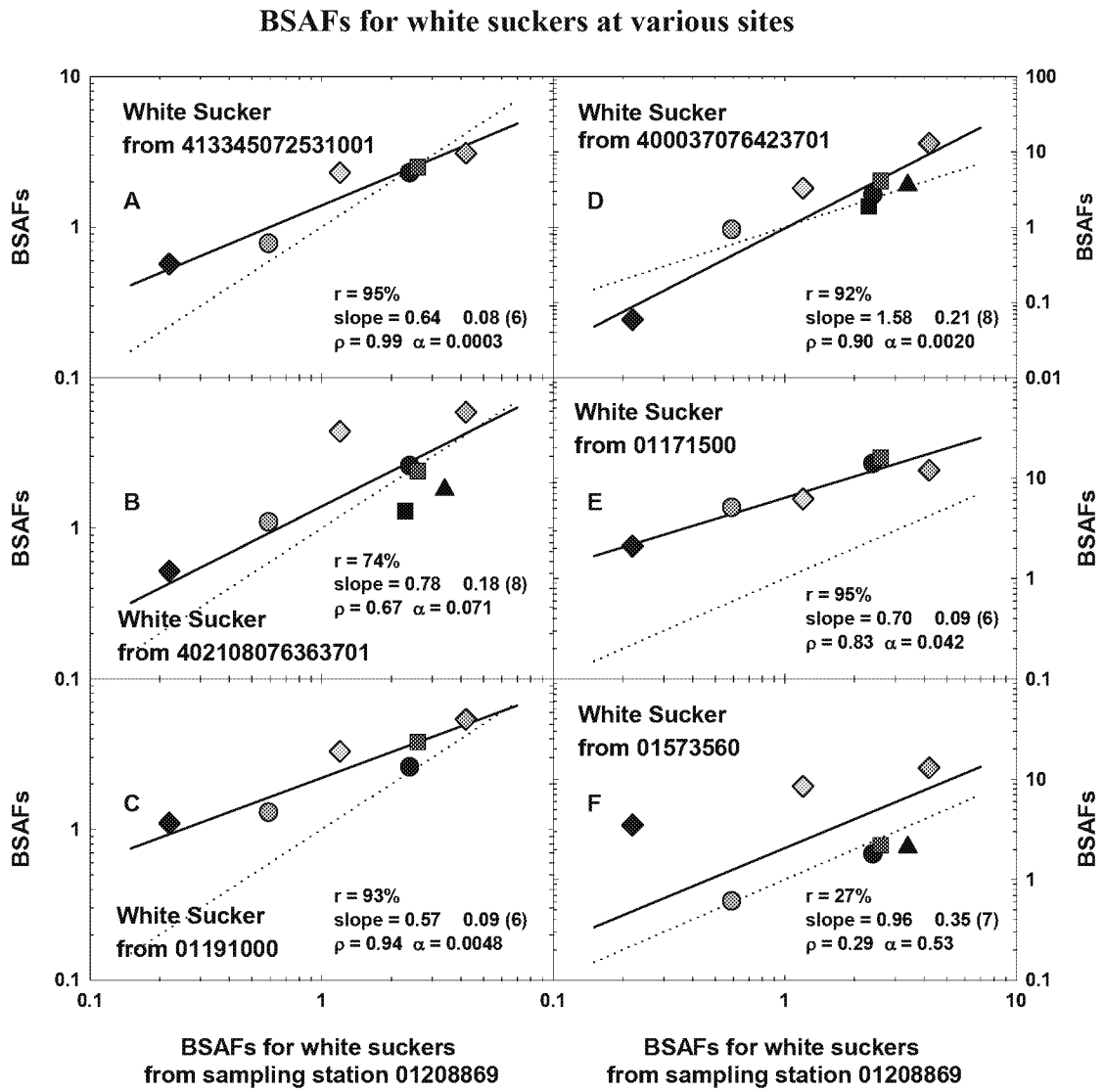

FIGURE 5-2. White sucker BSAFs (kg organic carbon/kg lipid) from six ecosystems plotted against BSAFs (kg organic carbon/kg lipid) for white sucker from sampling station 01208869 for *p,p*'-DDD (turquoise diamond symbols), *p,p*'-DDT (brown diamond), *p,p*'-DDE (green diamond), cis-chlordane (blue circle), trans-chlordane (orange circle), trans-nonachlor (purple square), dieldrin (blue triangle), and cis-nonachlor (blue square) (*4*). The sampling locations are those reported by Wong et al. (2001). The correlation coefficient (*r*), slope (standard deviation, number of data points) for geometric mean regression line (solid), Spearman's coefficient of rank correlation ( ) and significance level ( ), and 1:1 line (dotted) are provided. Note: the y-axes have different scales in some of the subgraphs.

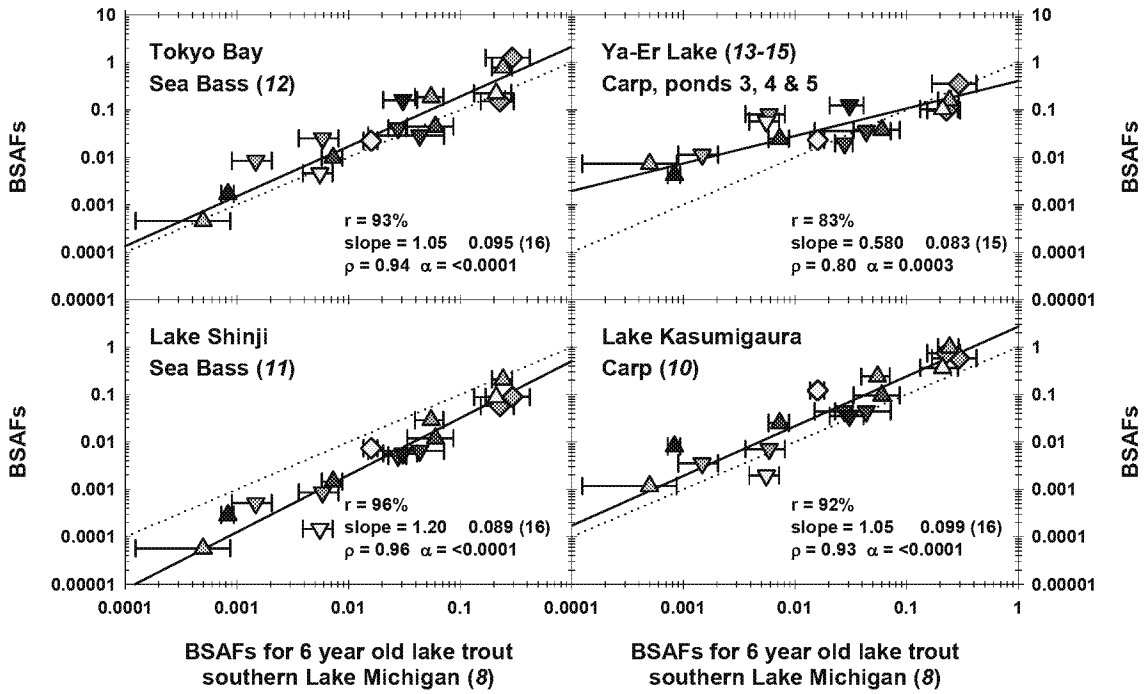

FIGURE 5-3. BSAFs (kg organic carbon/kg lipid) for PCDD/Fs with nonzero mammalian toxicity equivalence factors (TEFs) from four ecosystems plotted against BSAFs (kg organic carbon/kg lipid) for 6 year old lake trout from Lake Michigan. The symbol-color combination represents the same chemical in all four subgraphs, i.e., 2,3,7,8-TeCDD (green diamond); 1,2,3,7,8-PeCDD (orange diamond); 1,2,3,4,7,8-HxCDD (yellow diamond); 1,2,3,6,7,8-HxCDD (pink up triangle); 1,2,3,7,8,9-HxCDD (purple up triangle); 1,2,3,4,6,7,8-HpCDD (blue up triangle), OCDD (turquoise up triangle); 2,3,7,8-TeCDF (green up triangle); 1,2,3,7,8/1,2,3,4,8-PeCDF (orange up triangle); 2,3,4,7,8-PeCDF (yellow up triangle); 1,2,3,4,7,8/1,2,3,4,7,9-HxCDF (pink down triangle); 1,2,3,6,7,8-HxCDF (purple down triangle); 2,3,4,6,7,8-HxCDF (blue down triangle); 1,2,3,4,6,7,8-HpCDF (green down triangle); 1,2,3,4,7,8,9-HpCDF (orange down triangle); and OCDF (yellow down triangle). The correlation coefficient (*r*), slope (standard deviation, number of data points) for geometric mean regression line (solid), Spearman's coefficient of rank correlation ( ) and significance level ( ), and 1:1 line (dotted) are provided. 95% confidence limits on the Lake Michigan BSAFs are provided.

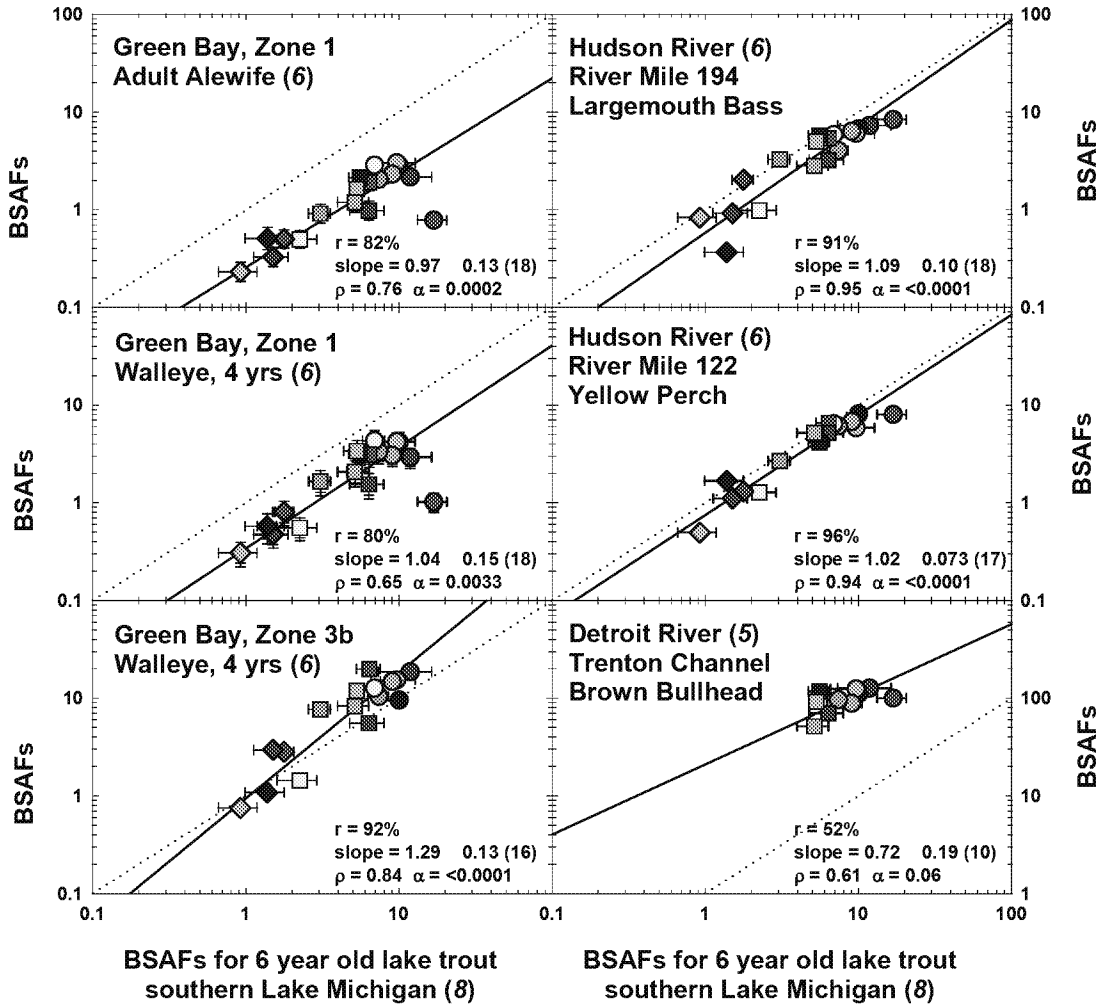

FIGURE 5-4. BSAFs (kg organic carbon/kg lipid) for PCBs from Green Bay, Hudson River, and Detroit River (Leadley et al., 1998) plotted against BSAFs (kg organic carbon/kg lipid) for 6 year old lake trout from Lake Michigan. The symbol-color combination represents the same chemical in all six subgraphs, i.e., PCB-18 (blue diamond), PCB-22 (turquoise diamond), PCB-26 (yellow square), PCB-28/31 (purple diamond), PCB-49 (purple square), PCB-52 (green square), PCB-56/60 (pink diamond), PCB-66 (orange square), PCB-85 (yellow circle), PCB-87 (orange circle), PCB-91 (pink square), PCB-97 (turquoise square), PCB-99 (blue circle), PCB-118 (blue square), PCB-141 (turquoise circle), PCB-146 (pink circle), PCB-149 (green circle), and PCB-180 (purple circle). The correlation coefficient (*r*), slope (standard deviation, number of data points) for geometric mean regression line (solid), Spearman's coefficient of rank correlation ( ) and significance level ( ), and 1:1 line (dotted) are provided. 95% confidence limits are provided for each BSAF when available.

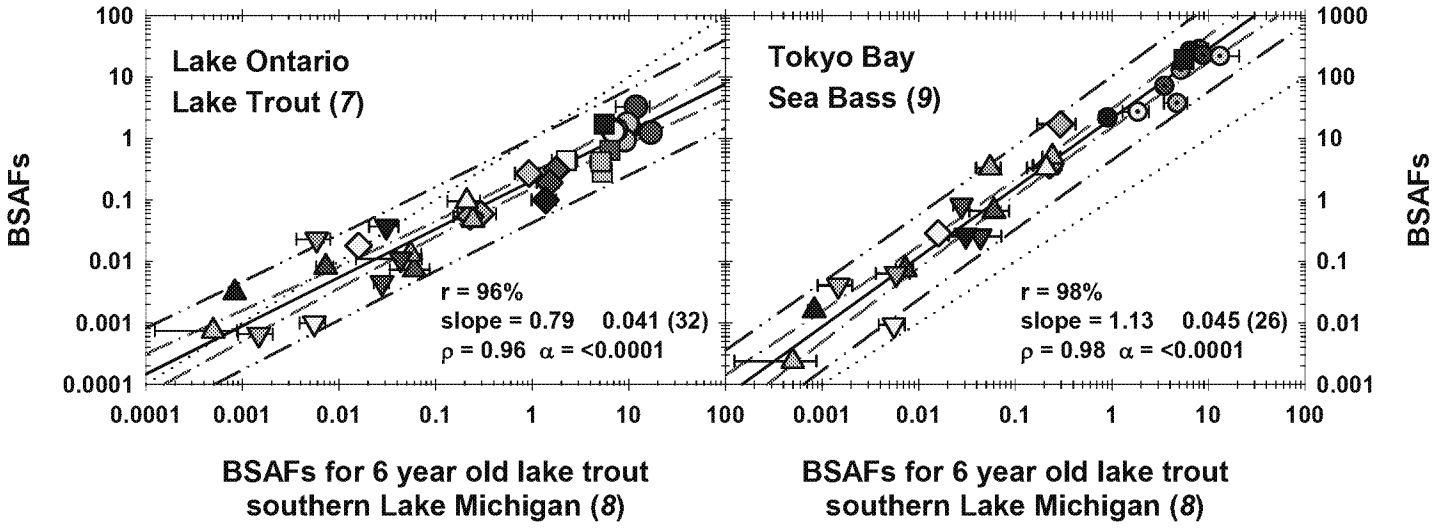

FIGURE 5-5. BSAFs (kg organic carbon/kg lipid) for PCBs and PCDD/Fs from Lake Ontario (USEPA, 1995) and Tokyo Bay (Naito et al., 2003) plotted against BSAFs (kg organic carbon/kg lipid) for 6 year old lake trout from Lake Michigan. The symbol-color combination represents the same chemical in both subgraphs, and their descriptions are listed in Figures 5-2 and 5-3. The correlation coefficient (*r*), slope (standard deviation, number of data points) for geometric mean regression line (solid), 95% confidence and prediction limits for the regression, Spearman's coefficient of rank correlation ( ) and significance level ( ), and 1:1 line (dotted) are provided. 95% confidence limits are provided for each BSAF when available. The Tokyo Bay data set had only one PCB in common with the PCBs used in Figure 5-3. The other nine PCBs with nonzero TEFs were plotted against BSAFs for Lake Michigan 6 year old lake trout, i.e., all dotted circles: PCB-77 (brown), PCB-81 (yellow), PCB-105 (orange), PCB-114 (blue), PCB-123 (pink), PCB-126 (purple), PCB-156 (turquoise), PCB-167 (red), and PCB 169 (green).

5.1.1 Estimating Site-Specific BAFs by Extrapolating BSAFs (Method 3a)

Method 3a estimates the site-specific baseline BAF by extrapolating the BSAF determined for the chemical of interest by measurements in another ecosystem, which is then multiplied by the sediment-water concentration quotient ($_{socw}$) for the site to obtain a trophic level-specific baseline BAF:

$$\text{Baseline BAF}_i = \text{BSAF}_{i,j}\ \Pi_{socw} - \frac{1}{f_l} \qquad \text{(Equation 5-1)}$$

The terms in this equation are defined as follows:

Baseline BAF_i	=	Baseline BAF estimated for the site and the chemical of interest, for organism at trophic level *i* (defined in Equation 3-2);
$BSAF_{i,j}$	=	BSAF for chemical of interest for organism at trophic level *i* extrapolated from ecosystem *j* (defined in Equation 4-1);
Π_{socw}	=	Sediment-water concentration quotient for the site and the chemical of interest (defined in Equation 4-3);
f_l	=	Lipid content (fraction) of the target species or tissue.

In most cases, $_{socw}$ will be unknown for the chemical of interest, because this coefficient is based on the chemical concentration measured in water, which is usually undetectable when Method 3 is selected to estimate BAFs. Therefore, the sediment-water concentration quotient for a reference chemical is usually substituted in equation 5-1, similar to the way $_{socw}$ for reference

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chemicals are used in Method 2. Equation 5-2 is used to calculate the site-specific baseline BAF for chemical *k*, when $_{socw}$ is based on measurements for reference chemicals *r*:

$$\text{Baseline BAF}_{i,k} = \text{BSAF}_{i,j} \frac{D_{k/r}\ \Pi_{socw,r}\ K_{ow,k}}{K_{ow,r}} - \frac{1}{f_l} \qquad \text{(Equation 5-2)}$$

where:

$D_{k/r}$ = Ratio of the fugacity gradient (modeled as $_{socw}/K_{ow}$) between sediment and water for chemical of interest *k* in comparison to that of a reference chemical *r*

Each of the parameters in equations 5-1 and 5-2 (BAF, BSAF and $_{socw}$) is calculated using chemical concentrations normalized for lipid (in biota) and/or organic carbon (sediment) contents and adjusted for the dissolved fraction of the chemical in water. Method 3a is appropriate for moderate to highly hydrophobic nonionic organic chemicals, and to certain ionic organic chemicals for which similar lipid and organic carbon partitioning behavior applies. Since a BSAF is based on lipid and organic carbon normalized chemical concentrations (Equation 4-1), no other adjustment for these factors is necessary. Equations 5-1 and 5-2 should only be applied to fish or other aquatic biota within a specific trophic level. Method 3a does not address site-specific variability in BSAFs. The only site-specific adjustment made by the investigator using Method 3a involves selecting a value for $_{socw}$, based upon measurements, estimates or predictions, as discussed in Section 4.3. Calculating a site-specific BAF using Method 3a is presented in the following example.

Extrapolating a Site-Specific BAF From BSAFs Determined at Another Site (Method 3a)

In this example, high-quality BSAF measurements from Lake Michigan are used to derive site-specific BAFs for a number of PCB congeners in adult (4 year old) walleye in Green Bay. The site is defined to be the middle portion of Green Bay, corresponding to sampling zone 3a from the Green Bay Mass Balance Study. Walleye is a popular sport fish, commonly caught and consumed by the local community. The dietary preference of adult walleye, based upon gut content analyses, places this species in trophic level 4.

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Extrapolating a Site-Specific BAF From BSAFs Determined at Another Site (Method 3a, continued)

The reference BSAFs used in this example was obtained from data published by Burkhard et al. (2004) for PCBs, PCDDs, and PCDFs from a study conducted in southern Lake Michigan that was specifically designed to determine BSAFs in multiple age classes (2, 3, 5-6 and 8-9 year old) of lake trout. The data were based upon highly representative sampling: southern Lake Michigan is well-mixed; fish samples consisted of 5 fish per composite sample and multiple composites per age class were analyzed; and sediments were sampled from five depositional areas surrounding the location where the fish were collected. Analyses of these data confirmed that the concentration measurements were both consistent and representative. The consistency in chemical-specific BSAFs determined in the Burkhard et al. (2004) study demonstrated that highly-reproducible BSAFs could be obtained from a site when appropriately sampled.

The chemical of interest is PCB 101, for which no BAF value has been determined at the site for the adult walleye target species. BSAFs for this PCB were determined in various age classes of lake trout in Lake Michigan by Burkhard et al. (2004). A review of the dietary preferences of the larger sizes of lake trout that are commonly consumed by the general U.S. population confirms that these organisms belong to trophic level 4. The BSAFs measured for PCB 101 in composite samples of large Lake Michigan lake trout are tabulated below; the geometric mean of the BSAFs is 7.71.

Lake Trout Composite Sample (age)	BSAF for PCB 101
6 year old	7.53
8 year old	6.40
9 year old	9.53

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Extrapolating a Site-Specific BAF From BSAFs Determined at Another Site (Method 3a, continued)

It is assumed that no sediment-water concentration quotient is available for the chemical of interest at the site, a common situation. Instead, $_{socw}$ measured at the site for reference chemicals will be used with Method 3a to estimate the site-specific baseline BAF. PCB congeners 52 and 105 have been used as reference chemicals for calculating baseline BAFs for other PCBs (USEPA, 1995a; Cook and Burkhard, 1995). These congeners serve as appropriate reference chemicals because (1) they have similar physicochemical properties, (2) they are well quantified in sediment and biota, and (3) available data indicate they have loading histories similar to PCB 101 and thus their fugacity ratio ($_{socw,r}/K_{ow,r}$) values should be similar. The sediment-water concentration quotient measured for PCBs 52 and 105 in Green Bay zone 3a are tabulated below.

PCB congener	$_{socw}$	log K_{ow}
52	4.24×10^{6}	5.84
105	2.37×10^{7}	6.65

Estimating the site-specific baseline BAF using method 3a

Equation 5-2 is used to calculate the site-specific baseline BAF for chemical i, when $_{socw}$ is based on measurements for reference chemicals r:

$$\text{Baseline BAF}_{i,k} = \text{BSAF}_{i,j}\,\frac{D_{k/r}\,\Pi_{socw,r}\,K_{ow,k}}{K_{ow,r}} - \frac{1}{f_l}$$

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Extrapolating a Site-Specific BAF From BSAFs Determined at Another Site (Method 3a, continued)

The K_{ow} of the chemical of interest, PCB 101, is 2.40×10^6; the lipid content of the target species, adult walleye, is 11%. Since the fugacity ratios of the chemical of interest and the reference chemicals are assumed to be similar, $D_{k/r}$ ~ 1. Therefore, a site-specific baseline BAF for PCB 101 is calculated using equation 5-2 with reference chemical PCB 52:

$$\text{Baseline BAF}_{4,101} = BSAF_{4,52}\frac{D_{101/52}\Pi_{SOCW,52}K_{OW,101}}{K_{OW,52}} - \frac{1}{f_l}$$

$$= (7.71)\frac{(1)(4.24\times10^6)(2.40\times10^6)}{(6.92\times10^5)} - \frac{1}{0.11} = 1.13\times10^8\, L/kg-l$$

Likewise, a site-specific baseline BAF for PCB 101 can also be calculated with reference chemical PCB 105:

$$\text{Baseline BAF}_{4,101} = BSAF_{4,105}\frac{D_{101/105}\Pi_{SOCW,105}K_{OW,101}}{K_{OW,105}} - \frac{1}{f_l}$$

$$= (7.71)\frac{(1)(2.37\times10^7)(2.40\times10^6)}{(4.47\times10^7)} - \frac{1}{0.11} = 9.82\times10^7\, L/kg-l$$

The final site-specific baseline BAF for the chemical of interest should be calculated as the geometric mean of the individual site-specific baseline BAFs calculated using the different reference chemicals. In this example, the final site-specific baseline BAF for PCB 101 estimated using Method 3a is therefore 1.06×10^8 L/kg-lipid.

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$D_{k/r}$, the ratio of the fugacity gradient ($_{socw}/K_{ow}$) for chemical of interest *k* in comparison to reference chemical *r*, is an especially important parameter in equation 5-2. Unfortunately, high quality datasets for $_{socw}$, from which $D_{k/r}$ can be calculated, are very limited. Selecting appropriate reference chemicals, and accurate values of $D_{k/r}$ is important, because significant reproducible differences in $_{socw}$ values between individual PCB, PCDD, PCDF, and PAH congeners are greater than previously recognized.

High-quality PCB congener data from southern Lake Michigan (Burkhard et al. 2004), 2 locations in Green Bay (Burkhard et al. 2003b), and 2 locations in the Hudson River (Burkhard et al. 2003b) will be used to further illustrate the Method 3a BSAF extrapolation methodology. These ecosystems are substantially different: Lake Michigan is a cold deepwater oligotrophic ecosystem, Green Bay is a shallow eutrophic ecosystem, and Hudson River is a relatively fast moving river ecosystem. Additionally, the aquatic food webs in the ecosystems are different and have different top predatory species: lake trout (*Salvelinus namaycush*) in Lake Michigan, brown trout (*Salmo trutta*) and walleye (*Stizostedion vitreum*) in Green Bay, and largemouth bass (*Micropterus salmoides*) and yellow perch (*Perca flavescens*) in Hudson River. For the Green Bay ecosystem, data used were from Zone 1, the lower Fox River entering into Green Bay, and Zone 4, the deeper outer portion of the bay. These are the most distinctly different two zones across the bay in terms of conditions and parameters, with Zone 1 having the highest concentrations of PCBs, and hydrodynamic and sediment transport dynamics characteristic of an urban river. In the Hudson River, two locations in Thompson Island Pool, river miles (RMs) 189 and 194, were used.

BSAF extrapolation involves directly applying measurements made in one ecosystem to the site of interest, as demonstrated in the following examples. In Table 5-1, PCB congener BSAFs measured in southern Lake Michigan for 6 year old trout were extrapolated to predator fish at multiple locations in the Green Bay and Hudson River ecosystems. In Table 5-1A and B, the Lake Michigan BSAFs are extrapolated to 3 year old brown trout and 4 year old walleye in Zone 4 of Green Bay, and in Table 5-1C the Lake Michigan BSAFs are extrapolated to 4 year old walleye in Zone 1 of Green Bay. In Tables 5-1 D and E, the Lake Michigan BSAFs are extrapolated to largemouth bass at Hudson River miles 189 and 194. In each sub-table, the BSAFs

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for 9 representative PCB congeners measured in Lake Michigan lake trout are extrapolated to the fish species indicated, and are used to estimate the site-specific BAF via Method 3a (equation 5-1). The Lake Michigan lake trout BSAFs are also compared to independently-determined BSAFs for each fish species at each site, and the error involved in BSAF extrapolation is presented in Table 5-1.

Comparison of the BSAF extrapolation errors in Table 5-1 suggest that Method 3a estimates tend to be consistently biased at each site. This is most apparent for the BSAFs extrapolated to Green Bay sites (Tables 5-1, A through C). BSAFs extrapolated to fish in zone 4 of Green Bay are negatively biased for all of the congeners included in the tabulation; the average bias is -53% for brown trout and -61 for walleye. BSAFs extrapolated to fish in zone 1 of Green Bay are positively biased for all of the congeners, with an average bias of +167% for walleye. To put this in context, if an investigator extrapolated Lake Michigan BSAFs to estimate site-specific BAFs for walleye in Green Bay Zone 4, the predicted BAFs would be too small by a factor of about 2.4. On the other hand, if the investigator used the Lake Michigan BSAFs to estimate site-specific BAFs for walleye in Zone 1 of Green Bay, the BAFs would be too large, by a factor of about 1.7. The errors in BSAFs extrapolated to Hudson River sites (Tables 5-1, D and E) appear to be more random, except for congener 18, which is highly biased at river mile 194. On an individual congener basis, the largest BSAF extrapolation errors were 304% for PCB 180 in Green Bay zone 1 walleye, and 275% for PCB 18 in largemouth bass at Hudson River mile 194. However, the majority of BSAF extrapolation errors were smaller than 100%. A graphical comparison between the measured and extrapolated BSAFs is presented in Figure 5-6. This figure demonstrates that the errors in BSAF extrapolation fall within the ± factor of 5 range (for this example) but that Method 3a extrapolation does not account for much of the site-specific variability in BSAFs. It should also be recognized that using PCB data does not fully demonstrate the benefits of Method 3a for the greater range of BSAF values for other potential chemicals of concern (e.g., TCDDs, TCDFs, PAHs), which can span up to several orders of magnitude.

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Table 5-1. Method 3a BSAF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River.

A. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to 3 Year Old Brown Trout in Zone 4 of Green Bay (GB BT3)

PCB Congener	Log K_{ow}	LM LT6 BSAF	GB BT3 BSAF	BSAF Extrapolation Error	socw Measured in Green Bay Zone 4	Predicted Site-specific Baseline BAF	Site-specific Log Baseline BAF
18	5.24	1.38	3.29	-58%	1.11×10^6	1.53×10^6	6.18
28/31	5.67	1.50	4.15	-64%	3.89×10^6	5.83×10^6	6.77
52	5.84	5.16	23.2	-78%	2.00×10^6	1.03×10^7	7.01
110	6.48	4.75	13.4	-65%	7.33×10^6	3.48×10^7	7.54
118	6.74	5.57	11.0	-49%	2.99×10^7	1.66×10^8	8.22
149	6.67	9.05	23.5	-61%	3.10×10^6	2.81×10^7	7.45
180	7.36	11.8	13.9	-15%	1.32×10^7	1.56×10^8	8.19
174	7.11	8.30	14.2	-42%	9.64×10^6	8.00×10^7	7.90
196/203	7.65	9.15	16.4	-44%	8.12×10^7	7.43×10^8	8.87

B. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to 4 Year Old Walleye in Zone 4 of Green Bay (GB W4)

PCB Congener	Log K_{ow}	LM LT6 BSAF	GB W4 BSAF	BSAF Extrapolation Error	socw Measured in Green Bay Zone 4	Predicted Site-specific Baseline BAF	Site-specific Log Baseline BAF
18	5.24	1.38	2.67	-48%	1.11×10^6	1.53×10^6	6.18
28/31	5.67	1.50	2.99	-50%	3.89×10^6	5.83×10^6	6.77
52	5.84	5.16	26.5	-81%	2.00×10^6	1.03×10^7	7.01
110	6.48	4.75	16.1	-70%	7.33×10^6	3.48×10^7	7.54
118	6.74	5.57	14.3	-61%	2.99×10^7	1.66×10^8	8.22
149	6.67	9.05	22.2	-59%	3.10×10^6	2.81×10^7	7.45
180	7.36	11.8	39.2	-70%	1.32×10^7	1.56×10^8	8.19
174	7.11	8.30	18.7	-56%	9.64×10^6	8.00×10^7	7.90
196/203	7.65	9.15	20.3	-55%	8.12×10^7	7.43×10^8	8.87

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Table 5-1 (Continued). Method 3a BSAF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River.

C. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to 4 Year Old Walleye in Zone 1 of Green Bay (GB W4)

PCB Congener	Log K_{ow}	LM LT6 BSAF	GB W4 BSAF	BSAF Extrapolation Error	socw Measured in Green Bay Zone 1	Predicted Site-specific Baseline BAF	Site-specific Log Baseline BAF
18	5.24	1.38	0.58	140%	1.57×10^{6}	2.16×10^{6}	6.34
28/31	5.67	1.5	0.47	216%	5.64×10^{6}	8.47×10^{6}	6.93
52	5.84	5.16	2.07	149%	3.98×10^{6}	2.06×10^{7}	7.31
110	6.48	4.75	2.05	131%	1.89×10^{7}	8.98×10^{7}	7.95
118	6.74	5.57	3.15	77%	4.87×10^{7}	2.71×10^{8}	8.43
149	6.67	9.05	3.06	196%	2.51×10^{7}	2.27×10^{8}	8.36
180	7.36	11.8	2.92	304%	1.68×10^{8}	1.99×10^{9}	9.30
174	7.11	8.3	3.70	124%	5.36×10^{7}	4.45×10^{8}	8.65
196/203	7.65	9.15	ND		ND		

D. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to Largemouth Bass at Hudson River mile 189 (RM 189 LMB)

PCB Congener	Log K_{ow}	LM LT6 BSAF	RM 189 LMB BSAF	BSAF Extrapolation Error	socw Measured at Hudson River Mile 189	Predicted Site-specific Baseline BAF	Site-specific Log Baseline BAF
18	5.24	1.38	1.29	7%	3.07×10^{6}	4.23×10^{6}	6.63
28/31	5.67	1.50	2.46	-39%	6.18×10^{6}	9.27×10^{6}	6.97
52	5.84	5.16	6.56	-21%	5.24×10^{6}	2.70×10^{7}	7.43
110	6.48	4.75	11.5	-59%	1.85×10^{7}	8.77×10^{7}	7.94
118	6.74	5.57	17.1	-67%	ND		
149	6.67	9.05	18.4	-51%	ND		
180	7.36	11.8	24.1	-51%	ND		
174	7.11	8.30	20.0	-59%	ND		
196/203*	7.65	9.15	30.4	-70%	ND		

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Table 5-1 (Continued). Method 3a BSAF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River.

E. Extrapolating Lake Michigan Lake Trout (LM LT6) BSAFs to Largemouth Bass at Hudson River mile 194 (RM 194 LMB)

PCB Congener	Log K_{ow}	LM LT6 BSAF	RM 194 LMB BSAF	BSAF Extrapolation Error	socw Measured at Hudson River Mile 194	Predicted Site-specific Baseline BAF	Site-specific Log Baseline BAF
18	5.24	1.38	0.368	275%	1.04×10^{7}	1.43×10^{7}	7.16
28/31	5.67	1.50	0.921	63%	1.60×10^{7}	2.40×10^{7}	7.38
52	5.84	5.16	2.82	83%	1.60×10^{7}	8.25×10^{7}	7.92
110	6.48	4.75	4.11	15%	5.32×10^{7}	2.52×10^{8}	8.40
118	6.74	5.57	5.77	-3%	ND		
149	6.67	9.05	6.36	42%	ND		
180	7.36	11.8	7.28	62%	ND		
174	7.11	8.30	5.23	59%	ND		
196/203*	7.65	9.15	8.92	3%	ND		

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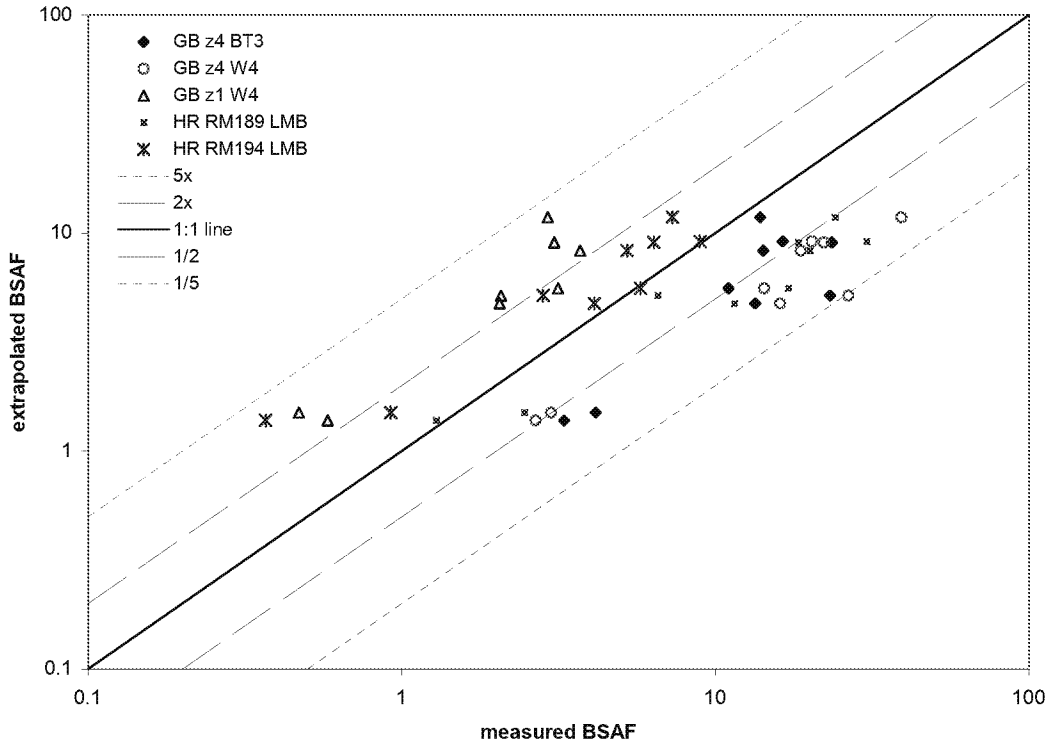

FIGURE 5-6. BSAFs (kg organic carbon/kg lipid) for PCBs from Green Bay and the Hudson River plotted against BSAFs (kg organic carbon/kg lipid) extrapolated from Lake Michigan (6 year old lake trout) using Method 3a. The symbol-color combinations represent particular fish species and ecosystem locations. GB = Green Bay; z1 = zone 1; z4 = zone 4; RM 189 = Hudson River mile 189; RM 194 = Hudson River mile 194; BT3 = 3-year old brown trout; W4 = 4-year old walleye; CP1 = 1-year old carp; LMB = largemouth bass; YP = yellow perch. The 1:1 line (solid), as well as ±2x (short dashed) and ± 5x (long-short dashed) lines are also provided.

5.1.2 Estimating Site-Specific BAFs by Extrapolating BEFs (Method 3b)

Method 3b estimates the site-specific baseline BAF by extrapolating a high quality BEF (a ratio of BSAFs) determined by measurements for the chemical of interest and a reference chemical in another ecosystem. The BEF is multiplied by a BSAF measured at the site for the reference chemical and the $_{socw}$ for the chemical of interest *k* at the site:

$$\text{Baseline BAF}_i = \text{BSAF}_{i,r}\ \text{BEF}_{j,k/r}\ \Pi_{socw,k} - \frac{1}{f_l} \qquad \text{(Equation 5-3)}$$

As was the case for Method 3a, $_{socw}$ will usually be unknown for the chemical of interest. Therefore, the sediment-water concentration quotient for a reference chemical is usually substituted in equation 5-3, similar to the way $_{socw}$ for reference chemicals are used in Method 2. Equation 5-4 is used to calculate the site-specific baseline BAF for the chemical of interest *k*, when $_{socw}$ is based on measurements for reference chemicals *r*:

$$\text{Baseline BAF}_i = \text{BSAF}_{i,r}\ \text{BEF}_{j,k/r}\ \frac{D_{k/r}\ \Pi_{socw,r}\ K_{ow,k}}{K_{ow,r}} - \frac{1}{f_l} \qquad \text{(Equation 5-4)}$$

As was the case for Method 3a (equation 5-2), the parameter $D_{k/r}$ plays an important role in equation 5-4.

The bioaccumulation equivalency factor $BEF_{j,k/r}$ between the chemical of interest *k* and the reference chemical *r* at another site (j) is:

$$\text{BEF}_{j,k/r} = \frac{\text{BSAF}_{j,k}}{\text{BSAF}_{j,r}} \qquad \text{(Equation 5-5)}$$

where:

$BSAF_{j,k}$	=	BSAF for chemical of interest *k* determined by measurements at another site *j*, and
$BSAF_{j,r}$	=	BSAF for reference chemical *r* determined by measurements at another site *j*.

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Although the terms in this equation have been previously defined, the investigator should note the use and meaning of the various subscripts associated with each term in equations 5-4 and 5-5:

$BSAF_{i,r}$	=	BSAF for reference chemical determined by measurements of organism at trophic level *i* at the site (equation 4-1);
$_{socw,k}$	=	Sediment-water concentration quotient for the site and the chemical of interest, *i* (defined in Equation 4-3).

As was the case for Method 3a, each of the parameters in equations 5-4 and 5-5 (BAF, BEF, BSAF and $_{socw}$) is calculated using chemical concentrations normalized for lipid (in biota) and/or organic carbon (sediment) contents and adjusted for the dissolved fraction of the chemical in water. Method 3b is appropriate for moderate to highly hydrophobic nonionic organic chemicals, and to certain ionic organic chemicals for which similar lipid and organic carbon partitioning behavior applies. Site-specific variability in BSAFs is addressed in Method 3b by incorporating a BSAF for a reference chemical determined by measurements at the site, as well as a site-specific value for $_{socw}$, based upon measurements, estimates or predictions (as discussed in Section 4.3). Equations 5-3 and 5-4 should only be applied to fish or other aquatic biota within a specific trophic level. Calculating a site-specific BAF using Method 3b is presented in the following example.

Extrapolating a Site-Specific BAF From BEFs Determined at Another Site (Method 3b)

In this example, Method 3b is used to extrapolate a BEF, or ratio of BSAFs, from Lake Michigan to the Green Bay site. In this case, the chemical of interest is PCB 89, for which no BAF value has been determined at the site for the adult walleye target species. As in the previous example, it is assumed that no sediment-water concentration quotient ($_{socw}$) is available for the chemical of interest at the site. PCB 52 was chosen as a reference chemical for this example (K_{ow}=6.92×10^5). Both $_{socw}$ (4.24×10^6) and a BSAF (5.67) have been determined for this PCB in Green Bay zone 3b. BSAFs for both of these PCBs were determined in various age classes of lake trout in Lake Michigan by Burkhard et al. (2004), as tabulated below.

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Extrapolating a Site-Specific BAF From BEFs Determined at Another Site (Method 3b, continued)

Lake Trout Composite Sample (age)	BSAF for PCB 89	BSAF for PCB 52
6 year old	4.80	5.16
8 year old	4.59	5.34
9 year old	6.21	6.90

Determining the bioaccumulation equivalency factor (BEF)

Method 3b extrapolates one or more bioaccumulation equivalency factors (BEFs) from another site j. The BEF is calculated using BSAFs for chemical of interest k and reference chemical r as:

$$\left(\mathrm{BEF}_j\right)_{\mathrm{k/r}} = \frac{\left(\mathrm{BSAF}_j\right)_{\mathrm{k}}}{\left(\mathrm{BSAF}_j\right)_{\mathrm{r}}} \qquad \text{(Equation 5-5)}$$

BEFs were calculated with BSAFs for individual lake trout composites; for example, the BEF based on PCB concentrations in 6 year old lake trout is:

$$\left(\mathrm{BEF}_{LM}\right)_{89/52} = \frac{\left(\mathrm{BSAF}_{LM,6yr}\right)_{89}}{\left(\mathrm{BSAF}_{LM,6yr}\right)_{52}} = \tfrac{4.80}{5.16} = 0.930$$

Likewise, the BEFs for 8 and 9 year old lake trout are 0.860 and 0.900, respectively, as tabulated below. The geometric mean of the three BEFs, 0.896, was the value extrapolated to Green Bay.

LAKE TROUT COMPOSITE SAMPLE (AGE)	BIOACCUMULATION EQUIVALENCY FACTOR (BEF)
6 year old	0.930
8 year old	0.860
9 year old	0.900

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Extrapolating a Site-Specific BAF From BEFs Determined at Another Site (Method 3b, continued)

Estimating the site-specific baseline BAF using method 3b

Equation 5-4 is used to calculate the site-specific baseline BAF for chemical k, when $_{socw}$ is based on measurements for reference chemical r and the $BEF_{j,k/r}$ is extrapolated from another site:

$$\text{Baseline BAF}_i = \text{BSAF}_{i,r}\text{BEF}_{j,k/r}\frac{D_{k/r}\Pi_{SOCW,r}K_{OW,k}}{K_{OW,r}} - \frac{1}{f_l}$$

The K_{ow} of the chemical of interest, PCB 89, is 1.17×10^6; the lipid content of the target species, adult walleye, is 11%. Since the fugacity ratios of the chemical of interest and the reference chemicals are assumed to be similar, $D_{k/r}$ ~ 1. A site-specific baseline BAF for PCB 89 is calculated using equation 5-4 with reference chemical PCB 52:

$$\text{Baseline BAF}_4 = \text{BSAF}_{4,52}\text{BEF}_{LM,89/52}\frac{D_{89/52}\ \Pi_{SOCW,52}\ K_{OW,89}}{K_{OW,52}} - \frac{1}{f_l}$$

$$= (5.67)(0.896)\frac{(1)(4.24\times10^6)(1.17\times10^6)}{(6.92\times10^5)} - \frac{1}{0.11} = 3.64\times10^7\ L/kg-l$$

Since only one reference chemical was used in this example, 3.64×10^7 L/kg-lipid is the final site-specific baseline BAF for PCB 89 estimated using Method 3b. As discussed in Section 5.1.3, EPA recommends repeating the Method 3a and 3b calculations using multiple reference chemicals, and then averaging the results.

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Method 3b offers the investigator two significant advantages in comparison to Method 3a. First, by extrapolating a BEF instead of a BSAF, Method 3b takes advantage the "relative scaling" phenomenon evident in BSAFs for multiple chemicals between sites, as discussed in Section 5.1. The relative ranking of BSAFs has been demonstrated to be more consistent than the BSAFs values themselves. Therefore, BEFs, which turn BSAF ranking into ratios, should also be highly consistent between sites. Secondly, there are practical advantages to Method 3b in terms of the BSAFs that are required to make the extrapolation (Equation 5-3). For many organic chemicals, BEFs will be available from high-quality datasets (e.g., Lake Michigan, Hudson River and Green Bay) as have been described in this and previous sections. Method 3b requires the investigator to determine the BSAF for a reference chemical at the site; however, the investigator can select the reference chemical based upon practical considerations such as analytical detectability. In many cases, PCBs will make good choices as references chemicals, because they can be readily quantified by available methods.

BEFs were introduced by EPA in the Great Lakes Water Quality Initiative (GLI) Technical Support Document (Cook and Burkhard, 1995; USEPA, 1995a) for use in estimating BAFs for PCDDs and PCDFs. Lake Ontario sediment and fish residue data (Lodge et al. 1994) provided the basis for calculating BEFs in the GLI. For example, table 5-2 illustrates the calculation of BEFs from lake-wide average concentrations of toxicologically important PCDDs and PCDFs in surface sediment and lake trout samples collected in 1987 for the EPA Region II Lake Ontario TCDD Bioaccumulation Study. Comparisons to BEFs calculated from data obtained for other ecosystems confirms these bioaccumulation potential differences and suggests that this BEF set would be predictive of bioaccumulation differences for PCDDs and PCDFs for fish in ecosystems outside the Great Lakes. This is important because very few PCDDs and PCDFs measured as sediment contaminants are also detectable in fish tissue. Based on the between-site comparisons of BSAFs presented in Section 5.1 (Figures 5-2 through 5-5), other persistent bioaccumulative organic chemicals such as PCBs and chlorinated pesticides also exhibit this behavior.

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Table 5-2. TCDD Bioaccumulation Equivalency Factors (BEFs) Derived For Toxicologically Important PCDDs And PCDFs From Lakewide Averages Of Concentrations In Lake Ontario Lake Trout And Surface Sediment In Depositional Areas.

Congener	Log K_{ow} [a,c]	BSAF	TCDD BEF
2,3,7,8-TCDD	7.02	0.059	1.0
1,2,3,7,8-PeCDD	7.50	0.054	0.92
1,2,3,4,7,8-HxCDD	7.80	0.018	0.31
1,2,3,6,7,8-HxCDD	7.80	0.0073	0.12
1,2,3,7,8,9-HxCDD	7.80	0.0081	0.14
1,2,3,4,6,7,8-HpCDD	8.20	0.0031	0.051
OCDD	8.60	0.00074	0.012
2,3,7,8-TCDF	6.5 [b]	0.047	0.80
1,2,3,7,8-PeCDF	7.0 [b]	0.013	0.22
2,3,4,7,8-PeCDF	7.0 [b]	0.095	1.6
1,2,3,4,7,8-HxCDF	7.5 [b]	0.0045	0.076
1,2,3,6,7,8-HxCDF	7.5 [b]	0.011	0.19
2,3,4,6,7,8-HxCDF	7.5 [b]	0.040	0.67
1,2,3,7,8,9-HxCDF	7.5 [b]	0.037	0.63
1,2,3,4,6,7,8-HpCDF	8.0 [b]	0.00065	0.011
1,2,3,4,7,8,9-HpCDF	8.0 [b]	0.023	0.39
OCDF	8.80	0.001	0.016

[a] Burkhard and Kuehl, 1987.
[b] Estimated based on degree of chlorination (Burkhard and Kuehl, 1987).
[c] EPA neither approves nor recommends the use of these log K_{ow} values, which were measured and/or estimated over 20 years ago, for use in deriving bioaccumulation factors. See Section 5.2.3.2 for guidance on selection of appropriate K_{ow} values.

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To further illustrate the estimation of BAFs using Method 3b, Lake Michigan lake trout BEFs for PCB congeners were extrapolated to brown trout and walleye in Zones 1 and 4 of Green Bay and to largemouth bass at RMs 189 and 194 of the Hudson River (Table 5-3). For these calculations, we chose to use PCB-118 as the reference chemical, so the BSAF for this congener was used to scale the Lake Michigan BEFs for each ecosystem. The product of the PCB-118 BSAF and the Lake Michigan BEFs provide the predicted BAFs. The choice of reference congener will affect the BAF estimate made using Method 3b; generally, more robust BAF estimates will be obtained by repeating the calculation using multiple reference chemicals and then averaging the results (Burkhard et al. 2003b). In each sub-table, the BSAFs for 9 representative PCB congeners measured in Lake Michigan lake trout are extrapolated to the fish species indicated, and are used to estimate the site-specific BAF via Method 3b (equation 5-3). In Table 5-3A and B, the Lake Michigan BEFs are extrapolated to 3 year old brown trout and 4 year old walleye in Zone 4 of Green Bay, and in Table 5-3C they are extrapolated to 4 year old walleye in Zone 1 of Green Bay. In Tables 5-3D and E, the Lake Michigan BEFs are extrapolated to largemouth bass at Hudson River miles 189 and 194. The Lake Michigan lake trout BSAFs are also compared to independently-determined site-specific BSAFs for each fish species at each site in Table 5-3, and the error involved in BSAF extrapolation is presented as well.

A graphical comparison between the measured and estimated BSAFs extrapolated from BEFs is presented in Figure 5-7. Comparison of the BSAF estimation errors for Method 3b (Table 5-3 and Figure 5-7) to those for Method 3a (Table 5-1 and Figure 5-6) demonstrate that, although the Method 3b estimates are not perfect, they are in much better agreement with the measured BSAF than the BSAFs which are directly extrapolated from one ecosystem to another (i.e., Method 3a). The average bias in Method 3b estimates ranges from a low of -8% for brown trout in Green Bay zone 4, to a high of 82% for largemouth bass at Hudson River mile 194. On an individual congener basis, variability in BEF extrapolation errors is again greater for the Hudson River sites than for those in Green Bay. In particular, large positive errors were calculated for PCB-18 for all species and locations on the Hudson River. It is not obvious why the extrapolation errors were so large for this congener. As was the case for Method 3a, the majority of the errors in BSAFs extrapolation by Method 3b were smaller than 100%, with the errors in BSAF extrapolation falling within the ± factor of 5 range (for this example).

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Table 5-3. Method 3b BEF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River.

A. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to 3 Year Old Brown Trout in Zone 4 of Green Bay (GB BT3)

PCB Congener	Log K_{ow}	LM LT6 BSAF	GB BT3 BSAF	BEF (PCB 118)	Method 3b BSAF Prediction	Measured GB BT3 BSAF	Method 3b BSAF Error	socw Measured in Green Bay Zone 4	Predicted Site-specific Log Baseline BAF
18	5.24	1.38		0.248	2.73	3.29	-17%	1.11×10^6	6.48
28/31	5.67	1.50		0.269	2.96	4.15	-29%	3.89×10^6	7.06
52	5.84	5.16		0.926	10.2	23	-56%	2.00×10^6	7.31
110	6.48	4.75		0.853	9.38	13	-30%	7.33×10^6	7.84
118	6.74	5.57	11.0	1.00				2.99×10^7	
149	6.67	9.05		1.62	17.9	24	-24%	3.10×10^6	7.74
180	7.36	11.8		2.12	23.3	14	68%	1.32×10^7	8.49
174	7.11	8.30		1.49	16.4	14	15%	9.64×10^6	8.20
196/203	7.65	9.15		1.64	18.1	16	10%	8.12×10^7	9.17

B. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to 4 Year Old Walleye in Zone 4 of Green Bay (GB W4)

PCB Congener	Log K_{ow}	LM LT6 BSAF	GB W4 BSAF	BEF (PCB 118)	Method 3b BSAF Prediction	Measured GB W4 BSAF	Method 3b BSAF Error	socw Measured in Green Bay Zone 4	Predicted Site-specific Log Baseline BAF
18	5.24	1.38		0.248	3.54	2.67	33%	1.11×10^6	6.59
28/31	5.67	1.50		0.269	3.85	2.99	29%	3.89×10^6	7.18
52	5.84	5.16		0.926	13.2	26.5	-50%	2.00×10^6	7.42
110	6.48	4.75		0.853	12.2	16	-24%	7.33×10^6	7.95
118	6.74	5.57	14.3	1.00				2.99×10^7	
149	6.67	9.05		1.62	23.2	22	5%	3.10×10^6	7.86
180	7.36	11.8		2.12	30.3	39	-23%	1.32×10^7	8.60
174	7.11	8.30		1.49	21.3	19	14%	9.64×10^6	8.31
196/203	7.65	9.15		1.64	23.5	20	16%	8.12×10^7	9.28

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Table 5-3 (Continued). Method 3b BEF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River.

C. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to 4 Year Old Walleye in Zone 1 of Green Bay (GB W4)

PCB Congener	Log K_{ow}	LM LT6 BSAF	GB W4 BSAF	BEF (PCB 118)	Method 3b BSAF Prediction	Measured GB W4 BSAF	Method 3b BSAF Error	socw Measured in Green Bay Zone 1	Predicted Site-specific Log Baseline BAF
18	5.24	1.38		0.248	0.78	0.575	36%	1.11×10^6	5.94
28/31	5.67	1.50		0.269	0.85	0.474	79%	3.89×10^6	6.52
52	5.84	5.16		0.926	2.92	2.07	41%	2.00×10^6	6.77
110	6.48	4.75		0.853	2.69	2.05	31%	7.33×10^6	7.29
118	6.74	5.57	3.15	1.00				2.99×10^7	
149	6.67	9.05		1.62	5.13	3.06	68%	3.10×10^6	7.20
180	7.36	11.8		2.12	6.68	2.92	129%	1.32×10^7	7.95
174	7.11	8.30		1.49	4.70	3.70	27%	9.64×10^6	7.66
196/203	7.65	9.15		1.64	5.18	ND		8.12×10^7	8.62

D. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to Largemouth Bass at Hudson River mile 189 (RM 189 LMB)

PCB Congener	Log K_{ow}	LM LT6 BSAF	RM 189 LMB BSAF	BEF (PCB 118)	Method 3b BSAF Prediction	Measured RM 189 LMB BSAF	Method 3b BSAF Error	socw Measured at Hudson River Mile 189	Predicted Site-specific Log Baseline BAF
18	5.24	1.38		0.248	4.22	1.29	228%	3.07×10^6	7.11
28/31	5.67	1.50		0.269	4.59	2.46	86%	6.18×10^6	7.45
52	5.84	5.16		0.926	15.8	6.56	141%	5.24×10^6	7.92
110	6.48	4.75		0.853	14.5	11.5	26%	1.85×10^7	8.43
118	6.74	5.57	17.1	1.00				ND	
149	6.67	9.05		1.62	27.7	18.4	50%	ND	
180	7.36	11.8		2.12	36.1	24.1	50%	ND	
174	7.11	8.30		1.49	25.4	20.0	27%	ND	
196/203	7.65	9.15		1.64	28.0	30.4	-8%	ND	

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Table 5-3 (Continued). Method 3b BEF Extrapolation Example Using PCB Data From Lake Michigan, Green Bay and the Hudson River.

E. Extrapolating Lake Michigan Lake Trout (LM LT6) BEFs to Largemouth Bass at Hudson River mile 194 (RM 194 LMB)

PCB Congener	Log K_{ow}	LM LT6 BSAF	RM 194 LMB BSAF	BEF (PCB 118)	Method 3b BSAF Prediction	Measured RM 194 LMB BSAF	Method 3b BSAF Error	socw Measured at Hudson River Mile 194	Predicted Site-specific Log Baseline BAF
18	5.24	1.38		0.248	1.43	0.368	289%	1.04×10^7	7.17
28/31	5.67	1.50		0.269	1.55	0.921	69%	1.60×10^7	7.39
52	5.84	5.16		0.926	5.34	2.82	89%	1.60×10^7	7.93
110	6.48	4.75		0.853	4.92	4.11	20%	5.32×10^7	8.42
118	6.74	5.57	5.77	1.00				ND	
149	6.67	9.05		1.62	9.37	6.36	47%	ND	
180	7.36	11.8		2.12	12.2	7.28	68%	ND	
174	7.11	8.30		1.49	8.60	5.23	64%	ND	
196/203	7.65	9.15		1.64	9.48	8.92	6%	ND	

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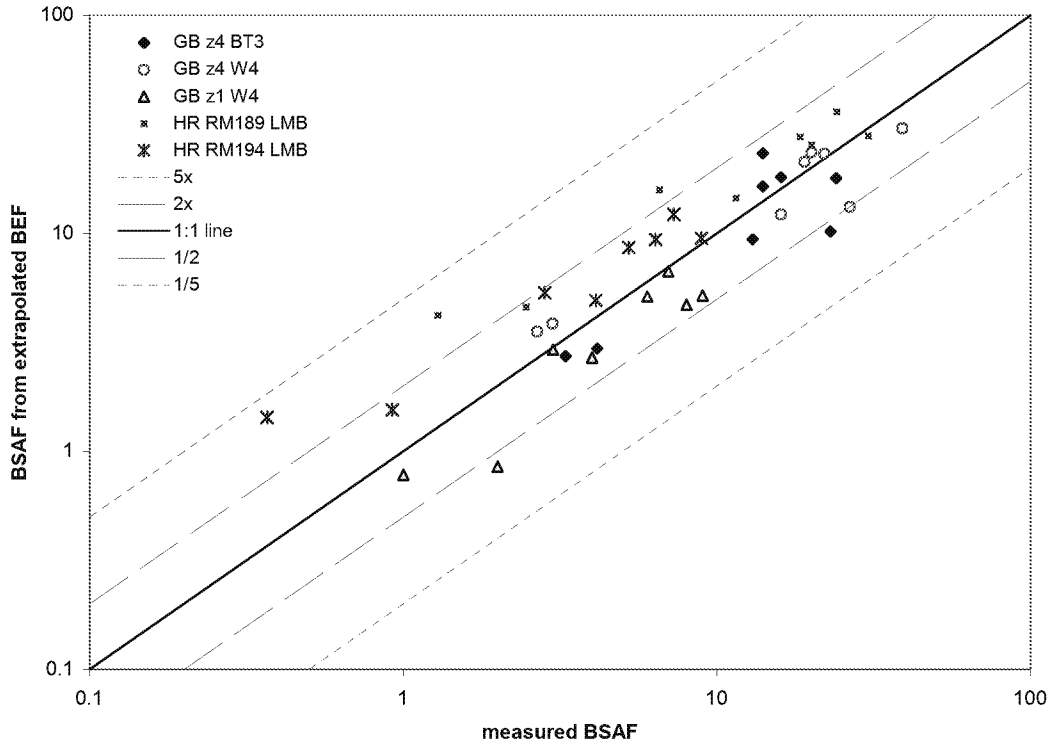

FIGURE 5-7. BSAFs (kg organic carbon/kg lipid) for PCBs from Green Bay and the Hudson River plotted against BSAFs (kg organic carbon/kg lipid) estimated from Lake Michigan (6 year old lake trout) BEFs using Method 3b. The symbol-color combinations represent particular fish species and ecosystem locations. GB = Green Bay; z1 = zone 1; z4 = zone 4; RM 189 = Hudson River mile 189; RM 194 = Hudson River mile 194; BT3 = 3-year old brown trout; W4 = 4-year old walleye; CP1 = 1-year old carp; LMB = largemouth bass; YP = yellow perch. The 1:1 line (solid), as well as ±2x (short dashed) and ± 5x (long-short dashed) lines are also provided.

5.1.3 How Can a Reference Site be Chosen?

To estimate a site-specific BAF by extrapolating a BSAF or BEF, BSAF measurements from an appropriate reference site are required. A good reference site is one where as many of the ecosystem criteria known to affect BSAFs (listed on page 5-7) as possible are similar to the site of interest. Therefore, the investigator should search for a reference site where the sediment-water column chemical distribution, degree of benthic food chain linkage, length of the food web, and bioavailability due to amounts and types of organic carbon are as similar as possible to the site of interest. Beyond these general considerations, there is relatively little guidance

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available regarding how best to choose a reference site with the goal of maximizing accuracy in extrapolation between sites. Some studies have demonstrated that a wide range of reference sites may be suitable. Sites where BSAFs were compared by Burkhard et al. (2005) and found to be generally compatible included large and small lakes, embayments, rivers and connecting channels.

Beyond ecosystem characteristics, better sites for BSAF or BEF extrapolation generally have higher numbers of samples for both biota and sediment, and sampling that provides for good spatial and temporal averaging. Ecosystems that are well-mixed in terms of contaminant concentrations will be easier to sample and should produce data that are simpler to average. Other aspects of quality assurance, such as good analytical techniques and sediment samples that are reflective of the actual exposure environment for the fishes are important, and have already been discussed in Sections 3.3 and 4.4.

If the investigator is considering BEF extrapolation (Method 3b), then the availability of a suitable reference chemical at the reference site should also be considered. Similarity of chemical properties (hydrophobicity, metabolism, persistence, etc.) between the chemical of interest and the reference chemical can affect the sensitivity of BEF extrapolation across species and sites. Selection of a suitable reference chemical (or chemicals) based upon the similarity of their properties to the chemical of interest is discussed in Section 4 (pages 4-5, 13 an 14).

If sufficient BSAF data are available for the site of interest and a potential reference site, the investigator can assess the compatibility between the sites by comparing the rank order of BSAFs for different chemicals at each site. As discussed in Section 5.1, consistent ordering/ranking of BSAFs across ecosystems may be a useful as an indicator of the validity of BSAF or BEF extrapolation.

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5.2 PREDICTING SITE-SPECIFIC BAFS USING BCFS AND FOOD CHAIN MULTIPLIERS (FCMs)

A site-specific BAF can be predicted as the product of a BCF coupled with a food chain multiplier (FCM). In effect, this method uses a simple bioaccumulation model to predict a site-specific BAF. The investigator has numerous options for predicting a site-specific BAF using this method. The BCF can be either a value based on laboratory experiments for the chemical of concern (Method 4a), or can be estimated using the K_{ow} of the chemical (Method 4b).

Site-Specific BAF Method 4

- Predicting BAFs using a BCF coupled with food chain multipliers, with 2 options:
  - 4a. Laboratory-measured BCFs
  - 4b. BCFs estimated using K_{ow}s

In addition, the investigator has the option to measure, estimate (from existing data), or predict (using food chain models) the FCM to reflect biomagnification of the chemical for a particular trophic level under site-specific conditions. Because food chain multipliers are trophic level-specific, the investigator should determine a FCM for each trophic level for which site-specific BAFs are being predicted using Method 4.

By definition, a BCF reflects only the accumulation of a chemical through the organisms' exposure to water. The BCF will likely underpredict BAFs for chemicals for which accumulation from sediment or dietary sources is important, including hydrophobic nonionic organic chemicals. Therefore, a FCM is used to adjust the value of a BCF to better account for chemical accumulation through the food web as a result of dietary exposures. For nonionic organic chemicals (and certain ionic organic chemicals to which similar lipid and organic carbon partitioning behavior applies), the food-chain multiplier is defined as the ratio of a baseline BAF for an organism of a particular trophic level to the lipid-normalized BCF (usually determined for organisms in trophic level one).

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5.2.1 Predicting Site-Specific Baseline BAFs using laboratory-measured BCFs and FCMs (Method 4a)

For Method 4a, a laboratory-measured BCF (BCF) and FCM are used to predict a site-specific baseline BAF. This method is applicable to nonionic organic chemicals that have moderate-to-high hydrophobicity (log K_{ow} 4) and low potential for being metabolized, and other chemicals that biomagnify. The BCF must be used in conjunction with an FCM because nonaqueous routes of exposure and subsequent biomagnification are of concern for these types of chemicals. Method 4a uses the following equation to calculate the baseline BAF for a site:

$$\text{Baseline BAF}_i = \text{FCM}_i \cdot \left[\frac{\text{BCF}_T^t}{\text{f}_{fd}} - 1 \right] \cdot \frac{1}{\text{f}_l} \qquad \text{(Equation 5-6)}$$

where:

BCF_T^t	=	Total BCF ($\text{BCF} = C_t/C_w$)
f_{fd}	=	fraction of the total concentration of chemical in BCF test water that is freely dissolved
f_l	=	fraction of the tissue that is lipid in the test organism
FCM_i	=	the food-chain multiplier for trophic level *i*, determined from appropriate field data or predicted for site-specific conditions

The baseline BAF and FCM in equation 5-6 are both trophic level-specific. The technical basis for Equation 5-6 is provided in Appendix A of TSD Volume 2 (USEPA, 2003). Guidance on selecting appropriate BCFs and FCMs, and the derivation of FCMs using food web models and field data, are provided below and discussed in greater detail in Section 5-3 of TSD Volume 2. Calculating a site-specific BAF using Method 4a is presented in the following example.

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Site-Specific BAF Predicted Using the Product of a Laboratory-Measured BCF and a FCM (Method 4a)

This example illustrates the prediction of a site-specific BAF for a trophic level 3 fish using Methods 4a for a nonionic organic chemical (chemical k). Calculating a site-specific BAF using Method 4a requires the investigator to use a laboratory-measured total BCF and a FCM.

Calculating a laboratory-measured BCF

Determination of a BCF requires information on the *total* concentration of chemical k in fish tissue and the *total* concentration of chemical k in the laboratory test water. Experimental data are available from an aquatic toxicology laboratory for the total concentration of chemical k in fish tissue (0.325 g/kg) and the laboratory test water (1.6 ng/L) in a water-only exposure test. The laboratory-measured BCF calculated for chemical k is 203 L/kg, as shown below:

$$\mathrm{BCF}_T^t = \frac{\mathrm{C}_t}{\mathrm{C}_w} = \frac{0.325\mu g}{kg} \cdot \frac{L}{1.6ng} \cdot \frac{1000ng}{\mu g} = 203\ L/kg$$

Determining a FCM Based on Measurements

As discussed in Section 5.2.2, site-specific FCMs can be determined by measurements or food chain model predictions. In this example, the FCM for trophic level 3 will be calculated from concentrations of chemical k measured in the food chain. The following data were obtained from field studies at the site:

Sample	Trophic level	Concentration of chemical k (g/kg)	Lipid or organic carbon (%)
Sediment	1	1.95	7.4
Phytoplankton	1	0.35	1.2
Zebra mussels	2	0.431	1.3
Crayfish	3	0.392	1.7

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Site-Specific BAF Predicted Using the Product of a Laboratory-Measured BCF and a FCM (Method 4a, continued)

Crayfish are frequently consumed by the local population, and were determined to be a preferred species at trophic level 3. At this site, crayfish consume zebra mussels (2/3 of diet by weight) and phytoplankton (1/3 of diet); zebra mussels consume phytoplankton (75%) and sediment (25%). Biomagnification factors (BMFs) can be calculated for trophic levels 2 and 3 using equations 5-11 and 5-12 and lipid-normalized chemical concentrations (the use of these equations is discussed in Section 5.2.2.1:

$$BMF_{TL2} = (C_{\ ,TL2}) / (C_{l,TL1}) \qquad \text{(Equation 5-11)}$$

$$BMF_{TL3} = (C_{l,TL3}) / (C_{l,TL2}) \qquad \text{(Equation 5-12)}$$

Since zebra mussels consume *both* phytoplankton and sediment, the BMF must be calculated using a weighted average of chemical concentrations in their diet items, sediment and phytoplankton:

$$BMF_{TL2(ZM)} = \frac{\frac{0.431\mu g}{kg} \cdot \frac{kg}{0.013kg - lipid}}{0.25 \cdot \left(\frac{1.95\mu g}{kg} \cdot \frac{kg}{0.074kg - SOC}\right) + 0.75 \cdot \left(\frac{0.35\mu g}{kg} \cdot \frac{kg}{0.012kg - lipid}\right)} = 1.16$$

Likewise, the BMF for crayfish must be calculated using the weighted average of chemical concentrations in their diet items, phytoplankton and zebra mussels:

$$BMF_{TL3(cf)} = \frac{\frac{0.392\mu g}{kg} \cdot \frac{kg}{0.017kg - lipid}}{(1/3) \cdot \left(\frac{0.35\mu g}{kg} \cdot \frac{kg}{0.012kg - lipid}\right) + (2/3) \cdot \left(\frac{0.431\mu g}{kg} \cdot \frac{kg}{0.013kg - lipid}\right)} = 0.725$$

FCMs can be calculated for trophic levels 2 and 3 using equations 5-8 and 5-9:

$$FCM_2 = BMF_2 = 1.16$$

$$FCM_3 = BMF_3 \quad BMF_2 = 0.725 \cdot 1.16 = 0.844$$

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Site-Specific BAF Predicted Using the Product of a Laboratory-Measured BCF and a FCM (Method 4a, continued)

Predicting a site-specific baseline BAF

The BCF_T^t is converted to a baseline BAF for a specific trophic level by incorporating information on the fraction of the chemical that is freely dissolved *in the bioconcentration test water* (f_{fd}), the fraction of tissue or aquatic organism tested that is lipid (f_l), and the site- and trophic level-specific FCM for the chemical. The site-specific baseline BAF is calculated from the BCF using equation 5-4:

$$\text{Baseline BAF}_i = \text{FCM}_i \cdot \left[\frac{\text{BCF}_T^t}{f_{fd}} - 1\right] \cdot \frac{1}{f_l}$$

Determining the fraction of chemical k that is freely dissolved in the bioconcentration test water (f_{fd}) requires information on the POC and DOC concentrations in the test water and the K_{ow} of chemical k.

For this example, the median POC concentration in the test water is 0.5 mg/L and the median DOC concentration is 10 mg/L. It is important that the POC and DOC concentrations used in calculating the freely dissolved fraction for baseline BAFs be determined from the water used in the BCF study. It is not appropriate to use site-specific POC and DOC concentrations to derive baseline BAFs from BCF_T^ts.

The K_{ow} for chemical k is 2×10^4, or a log K_{ow} of 4.3. Based on these data, the fraction of chemical k that is freely dissolved is 0.975, calculated using equation 3-12:

$$\left(f_{fd}\right)_{chemical\ i} = \frac{1}{1 + \frac{0.5mg - POC}{L} \cdot 2\times10^4 \frac{L}{kg} \cdot \frac{kg}{10^6 mg} + 0.08 \cdot \frac{10mg - DOC}{L} \cdot 2\times10^4 \frac{L}{kg} \cdot \frac{kg}{10^6 mg}} = 0.975$$

The f of the fish species sampled in the laboratory in this example was 2% (0.02). Using this f_l, the FCM measured at the site, and the BCF_T^t and f_{fd} calculated above, a site-specific baseline BAF of 8.8×10^3 L/kg-lipid is calculated as follows (Equation 5-4):

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Site-Specific BAF Predicted Using the Product of a Laboratory-Measured BCF and a FCM (Method 4a, continued)

$$\text{Baseline BAF}_3 = 0.844 \cdot \left[\frac{203\frac{L}{kg}}{0.975} - 1\right] \cdot \frac{1}{0.02} = 8.8\times10^3 L/kg - lipid$$

Calculating a site-specific total BAF

In order to determine a water quality standard for chemical k at the example site, the site-specific baseline BAF must be converted to a site-specific total BAF. The average POC concentration measured at the site is 0.54 mg/L, and the average DOC is 3.5 mg/L. The freely dissolved fraction of chemical k in the site water column can be calculated using equation 3-12:

$$f_{fd} = \frac{1}{1 + \frac{0.54mg - POC}{L} \cdot 2\times10^4 \frac{L}{kg} \cdot \frac{kg}{10^6 mg} + 0.08 \cdot \frac{3.5mg - DOC}{L} \cdot 2\times10^4 \frac{L}{kg} \cdot \frac{kg}{10^6 mg}} = 0.984$$

The lipid content for crayfish of harvestable size at the site is 1.7%. The site-specific total BAF can then be recalculated from the site-specific baseline BAF:

$$\text{Site-Specific BAF}_t^T = \left(0.017 \cdot 8.8\times10^3 \tfrac{L}{kg-l} + 1\right) \cdot 0.984 = 147 L/kg$$

The site-specific total BAF for chemical k in crayfish is 147 L/kg.

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Method 4a is based on the following assumptions: (1) a high-quality BCF is a reliable measure of the bioconcentration potential of a chemical in a particular species or trophic level of aquatic organism, (2) the measured BCF and the baseline BAF predicted with Method 4a are independent of chemical concentration in the water, and (3) FCMs account for biomagnification processes caused by the consumption of contaminated food in aquatic food webs.

Method 4a predictions address the effects of chemical metabolization on bioaccumulation, although BAFs predicted for metabolizing chemicals by this method may be inaccurate for a number of reasons. BCF for chemicals that are metabolized by the test organisms incorporate the effects of the metabolism on the concentration of chemical that is accumulated in the organism. However, if induction of metabolic systems is required, or co-occurring contaminants (i.e., that exist in the environment) are required for the metabolism to take place, then the effect of metabolism may not be captured in the BCF measurement. Therefore, the range of effects of metabolism on BCF will be chemical specific. Nevertheless, EPA believes that high-quality BCFs may provide a better measure of bioconcentration potential for chemicals than assuming that the lipid-normalized BCF is equal to the chemical's K_{ow} (i.e., Method 4b) because of the potential of the BCF to include the effects of metabolic processes. Furthermore, BCFs can be measured or obtained for specific species of interest. This specificity may reduce uncertainties associated with extrapolating bioaccumulation factors among species with known or suspected differences in metabolic pathways or capacity.

The baseline BAFs derived with Method 4a for chemicals that are metabolized will not include the effects of all metabolic processes because of the assumption of no metabolism used in deriving the FCMs (Table 5-6). However, the method will incorporate those metabolic processes or effects that are captured in the BCF measurement, and in field derived FCMs, when used. Baseline BAFs predicted from measured BCF for chemicals that are metabolized will be smaller than those predicted from measured BCFs for chemicals of equal hydrophobicity but which are not metabolized.

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A major limitation associated with Method 4a is the current lack of high-quality measured BCF data for highly hydrophobic chemicals in any organism class. This lack of data is due principally to the difficulties associated with performing BCF measurements for highly hydrophobic chemicals. Conditions appropriate for performing these measurements are described in Section 5.3.1 of TSD Volume 2. When evaluating BCF data in the literature, one often finds measurements performed with (1) conditions that do not meet current standards, for example, a solvent carrier such as acetone is used to introduce the chemical into the aqueous phase or the concentration in water exceeded the chemical's solubility, and (2) poor and/or incomplete reporting of measurement conditions and parameters, for example, no lipid data, no POC and DOC data, and/or an inability to determine whether steady-state conditions were obtained in the experiment. In addition, some BCFs were measured with chemical mixtures, such as Aroclors, and resolving the effects of co-occurring chemicals on micelle formation is often intractable. As BCF data become available for highly hydrophobic chemicals in the future, the impact of this limitation will lessen. Specific guidance for conducting BCF experiments or for reviewing studies for appropriate values to use in this method is provided in Section 5.3.1 of TSD Volume 2 (USEPA, 2003).

5.2.1.1 Validation of Method 4a

To date, EPA has performed only a limited number of evaluations of Method 4a because of a lack of BCF data of the appropriate quality. For example, EPA invested considerable effort in examining the scientific literature for measured BCF s for PCB congeners and was not able to find BCF s of appropriate quality.

Burkhard et al. (1997) evaluated Method 4a by using field data for chlorinated benzenes, butadienes, and hexachloroethane from Bayou d'Inde, Lake Charles, Louisiana. The results of this evaluation showed that field-measured baseline BAFs were within a factor of 3 for 88% and a factor of 5 for 94% of the baseline BAFs predicted using Method 4a (n = 32) (Figure 5-8). The median of the ratios of the field-measured baseline BAFs to predicted baseline BAFs was 1.03, and approximately one-half of the predicted baseline BAFs were less than the measured baseline BAFs (53%, n = 32). The chemicals whose field-measured baseline BAFs were in least

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agreement with the predicted baseline BAFs were hexachloroethane, Z-pentachlorobutadiene, and hexachlorobutadiene for *Callinectes sapidus* (blue crab). Metabolism of these chemicals by *C. sapidus* is suggested as the cause of the poor agreement between the field-measured BAFs and the baseline BAFs predicted using this method (Burkhard et al. 1997).

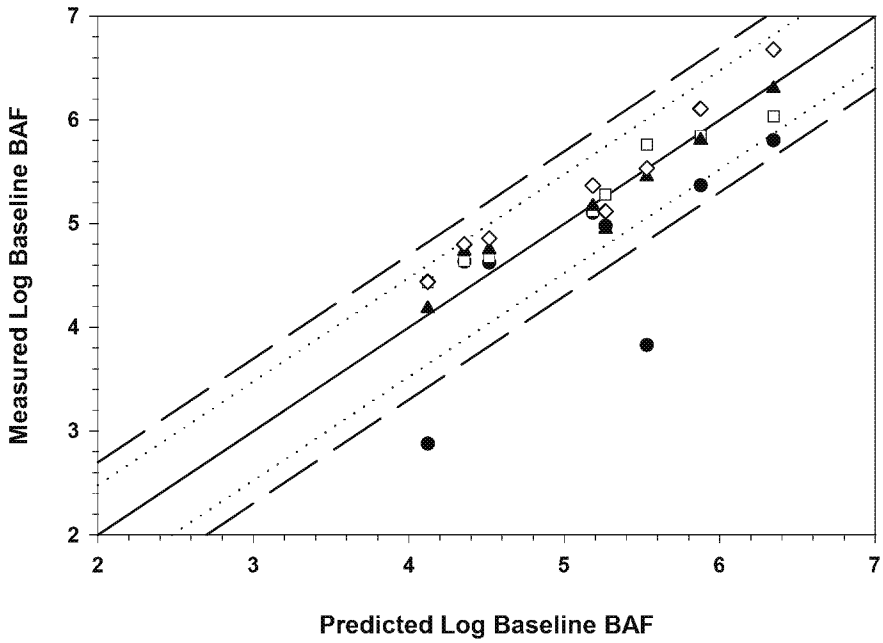

FIGURE 5-8. Relationship between baseline BAFs measured at Bayou d'Indie and BAFs predicted using Method 4a. The dotted and dashed lines represent a factor of 3 and 5 difference between the measured and predicted baseline BAFs, respectively. Baseline BAFs measured using *Callinectes sapidus* ( ), *Micropoganias undulatus* ( ), *Fundulus heteroclitus* ( ), and *Brevoortia patronus* ( ).

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5.2.2 Determining Site-Specific FCMs

FCMs are used in both Methods 4a and 4b to calculate the dietary transfer of a chemical. They represent a measure of the chemical's tendency to biomagnify in aquatic food webs. FCM values can range from 1 (no biomagnification) up to about 25 (significant biomagnification), depending upon the chemical, organism, and food web. Because FCMs can vary due to site-specific factors, the investigator should consider determining a FCM value that is most appropriate for use in estimating a baseline BAF using Method 4a or 4b. FCMs for a particular chemical, organism, and site can be determined using field data and/or a food web model. By definition, a FCM is:

(Equation 5-7)

$$\mathrm{FCM}_i = \frac{\text{Baseline BAF}_i}{\left[\dfrac{\mathrm{BCF}_T^t}{\mathrm{f}_{fd}} - 1\right] \cdot \dfrac{1}{\mathrm{f}_l}} = \frac{\text{Baseline BAF}_i}{\text{Baseline BCF}}$$

Equation 5-7 is simply a rearrangement of Equation 5-6. Calculating a food chain multiplier using Equation 5-7 is presented in the following example.

Calculation of food chain multipliers

This example illustrates the calculation of food chain multipliers for hexachlorobenzene (HCB) in trophic level 3 and 4 fish using equation 5-7. According to the Arnot and Gobas (2006) BCF/BAF database, several "acceptable" baseline BCF values have been measured for HCB. The geometric mean of these values is 415,000 L/Kg-lipid.

Baseline BAF values for HCB can be calculated for fish in the Lake Ontario ecosystem based on available data (Oliver and Niimi, 1988; Niimi and Oliver, 1989):

Fish	Trophic level	HCB concentration (ng/g)	Lipid content (%)
Alewife	3	20	7.0
Lake trout	4	90	17.4

Chemical concentrations in fish (C_l) were normalized by the lipid content (f_l) of each sample: $C_l = C_t / f_l$

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Calculation of food chain multipliers

The lipid-normalized chemical concentration in each sample is tabulated:

fish	HCB Concentration (ng/g-lipid)
Alewife	286
Lake Trout	517

These authors reported concentrations of HCB in Lake Ontario water to be 150 pg/L, with a DOC concentration of 2 mg/L. The freely dissolved fraction of chemical in the water column (f_{fd}) can be calculated using equation 3-6:

$$f_{fd} = 1 / (1 + POC \cdot K_{ow} + 0.08 \cdot DOC \cdot K_{ow})$$

The log K_{ow} for HCB is 5.73 (Arnot and Gobas, 2006), so $K_{ow} = 5.37 \times 10^5$. The water samples were centrifuged to remove particulates prior to extraction, so the POC concentration is (presumably) zero:

$$f_{fd} = \frac{1}{1 + 0.08 \cdot \frac{2\ mg - DOC}{L} \cdot 5.37 \times 10^5 \frac{L}{kg} \cdot \frac{kg}{10^6 mg}} = 0.92$$

The freely dissolved chemical concentration (C_w^{fd}) is calculated as:

$$C_w^{fd} = f_{fd} \cdot C_w = 0.92 \cdot 150 \text{ pg/L} = 138 \text{ pg/L}$$

Baseline BAFs were then calculated for alewife and lake trout, using Equation 3-2:

$$\text{Baseline BAF}_i = \frac{C_\ell}{C_w^{fd}} - \frac{1}{f_\ell}$$

$$\text{Baseline BAF}_3 = \frac{286 ng}{g\text{-}lipid} \cdot \frac{L}{138 pg} \cdot \frac{1000 pg}{1 ng} \cdot \frac{1000 g}{kg} - \frac{1}{0.07} = 2.07 \times 10^6\ L / kg - lipid$$

$$\text{Baseline BAF}_4 = \frac{517 ng}{g\text{-}lipid} \cdot \frac{L}{138 pg} \cdot \frac{1000 pg}{1 ng} \cdot \frac{1000 g}{kg} - \frac{1}{0.174} = 3.74 \times 10^6\ L / kg - lipid$$

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Calculation of food chain multipliers (continued)

Equation 5-7 can then be used to calculate FCMs for alewife and lake trout:

$$FCM_i = \frac{\text{Baseline BAF}_i}{\text{Baseline BCF}}$$

$$FCM_3 = 2.07 \times 10^6 \,/\, 4.15 \times 10^5 = 5.0$$

$$FCM_4 = 3.74 \times 10^6 \,/\, 4.15 \times 10^5 = 9.0$$

Based on these data for baseline BCF and baseline BAFs, the trophic level 3 food chain multiplier for HCB is 5.0 and the trophic level 4 FCM is 9.0.

In effect, equation 5-7 says that the FCM is the ratio between the chemical accumulation via all relevant routes (aqueous, dietary, and sediment) and the chemical bioconcentrated via aqueous exposure only. When the BCF is lipid normalized and corrected for growth dilution and bioavailability considerations (i.e., a baseline BCF), then the FCM will be relatively constant under steady-state conditions. Because a BCF is determined by using a water-only exposure to the chemical, it represents a trophic level 1 exposure for the organisms. When organisms occupy higher trophic levels in food webs, concentrations of many hydrophobic organic chemicals in their tissues will exceed those that are due to water exposure only, because of dietary uptake of the chemical. The FCM for the organism's trophic level accounts for the influences of dietary uptake by the organism. Dietary uptake of the chemical generally becomes important when the chemical's hydrophobicity exceeds a log K_{ow} of 4 and the rate of chemical metabolism by the organism is small.

5.2.2.1 Measuring Site-Specific FCMs

Field data can be used to derive FCMs for nonionic organic chemicals. FCMs derived from field measurements incorporate the conditions existing at the site where the measurements are performed. This includes the existing disequilibrium, chemical metabolism, and influences

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due to the structure of the food web (i.e., predator-prey relationships and benthic-pelagic components). FCMs derived from field measurements also account for any metabolism of the pollutant of concern by the aquatic organisms used to calculate the FCM.

Specifically, FCMs can be derived from chemical concentrations measured in the target organism and in organisms at each lower trophic level in the organism's food web. Field-derived FCMs should be calculated with lipid-normalized concentrations of the nonionic organic chemical measured at the site, in appropriate predator and prey species, using the following equations:

$$FCM_2 = BMF_2 \qquad \text{(Equation 5-8)}$$

$$FCM_3 = BMF_3 \quad BMF_2 \qquad \text{(Equation 5-9)}$$

$$FCM_4 = BMF_4 \quad BMF_3 \quad BMF_2 \qquad \text{(Equation 5-10)}$$

where:

FCM_i = food chain multiplier for trophic level *i* and

BMF_i = Biomagnification factor for trophic level *i*.

The basic difference between FCMs and BMFs is that FCMs relate back to trophic level 1, whereas BMFs always relate back to the next lowest trophic level. For nonionic organic chemicals, BMFs can be calculated from lipid-normalized concentrations of chemical in tissues of biota at a site according to the following equations:

$$BMF_2 = C_{,2} / C_{l,1} \qquad \text{(Equation 5-11)}$$

$$BMF_3 = C_{l,3} / C_{l,2} \qquad \text{(Equation 5-12)}$$

$$BMF_4 = C_{l,4} / C_{l,3} \qquad \text{(Equation 5-13)}$$

where:

$C_{l,i}$ = lipid-normalized concentration of chemical in tissue or whole organism at a specified trophic level (i = 2, 3, or 4).

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Examples of applying equations 5-8 through 5-13 were presented in the Method 4A example.

In addition to the guidance offered in Section 3.3 for determining baseline BAFs based on measurements made at the site, the following procedural and quality assurance guidelines apply to field-measured FCMs.

1. Information should be available to identify the appropriate trophic levels for the aquatic organisms and appropriate predator-prey relationships for the site for which FCMs are being determined. Information about trophic status is most accurate when obtained from the site(s) of interest, because predator-prey relationships for some species can vary widely over space and time. When a predator species consumes multiple prey species at a particular trophic level, chemical concentrations in prey species should be appropriately weighted (if the data are available) when used to calculate field-based FCMs. A number of approaches are commonly applied to determine trophic levels of aquatic organisms, including shifts in stable isotope ratios (e.g., ratios for C, N, and S are reported as ^{13}C, ^{15}N, and ^{34}S, respectively; Peterson and Fry, 1987; Jardine et al., 2006) and analysis of gut contents. Ratios of stable isotopes can change between diet and consumer due to differential digestion or fractionation during assimilation and metabolic processes. Metabolic fractionation also may cause isotope ratios of different tissues to vary substantially within individual consumers (McCutchan et al. 2003). General information on determining trophic levels of aquatic organisms can be found in USEPA 2000 a-c.

2. The aquatic organisms sampled from each trophic level should reflect the most important exposure pathways leading to human exposure via consumption of aquatic organisms. For higher trophic levels (e.g., 3 and 4), aquatic species used to calculate FCMs should be those that are commonly consumed by humans. The species sampled should also reflect size and age ranges that are typical of human consumption patterns at the site.

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3. The study from which the FCMs are derived should contain enough supporting information to determine that tissue samples were collected and analyzed according to appropriate, sensitive, accurate, and precise methods.

4. The percent of tissue that is lipid should be either measured or reliably estimated for the tissue(s) used to determine the FCM.

5. The chemical concentrations in the tissues/organisms used to calculate FCMs should reflect long-term average exposures of the target species to the chemical of interest; longer averaging periods are generally necessary for chemicals with greater hydrophobicity.

5.2.2.2 Predicting FCMs using a food chain model

Food chain model predictions can also be used to derive FCMs for nonionic organic chemicals. EPA applied the Gobas food chain model (Gobas, 1993) to predict FCMs as a function of trophic level and chemical hydrophobicity for the National BAF Methodology (USEPA, 2003). For that application, EPA selected and applied representative values for the various input parameters to that model and calculated FCMs for trophic levels 2, 3 and 4 for a mixed benthic-pelagic food web. EPA recognized that the food chain modeling approach could also be used to predict FCMs for conditions and parameters at a particular site, which could be different from the representative values used in the national methodology calculations. FCMs predicted using site-specific conditions and parameters will likely differ from the FCMs predicted in the National BAF Methodology to the extent that site conditions and parameters differ from the nationally-representative conditions.

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In deriving FCMs using a food web model, the investigator assumes that (1) the model is valid for the particular chemical, aquatic organism, food web and site, and (2) appropriate values are selected for all necessary model inputs. In other words, the investigator is responsible for selecting both a model and its input parameters. This section discusses how EPA selected a food web model for use in the 2000 Human Health Methodology. Also described are the parameters used with the model: the food web structure, $_{\text{socw}}$ (or, equivalently, C_w^{fd} and C_{soc}), and the chemical metabolism rate in the various organisms of the food web. Although data on the metabolism of most chemicals is currently quite limited, when available this information should be considered and potentially used. The Gobas (1993) model, for example, allows the user to input a metabolic transformation rate constant. Because all food web models require the above input parameters, these inputs are not unique to the food web model selected by EPA.

For a food web model to provide useful predictions, it should have the following general characteristics and qualities. First, the model should provide a full and complete description of the bioaccumulation process. Specifically:

- All biotic components of the food web must be represented: plankton, benthic invertebrates, forage fish, and piscivorous fish.
- It should account for chemical uptake and loss from both food and water for all organisms.
- It should include chemical concentrations in sediment and the water column, because these environmental compartments are the primary exposure media for benthic invertebrates and phytoplankton, respectively, and these organisms reside at the base of the benthic and pelagic food web.

In addition, steady-state solutions for predicting bioaccumulation in the food chain model are preferred over time-variant dynamic solutions for the food chain model, because AWQCs for the protection of human health are designed for long-term average conditions in ambient waters. Other desirable qualities include (1) the model is easy to run by the average user, (2) the model does not mix fate and transport models with the food chain model, (3) the model code does not require substantial validation each time it is used, and (4) the model parameters and other inputs can be readily measured or estimated.

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Although these attributes can make a food chain model relatively easy to use, the accuracy or uncertainty of model predictions depends largely upon *how* they are applied. Food chain model predictions may be highly uncertain unless they are confirmed by data (i.e., chemical concentrations in the modeled organisms). Burkhard (1998) determined that the uncertainty of food chain model predictions due to parameter variability and error, for a very well-studied ecosystem (i.e., PCBs in the Lake Ontario salmonid food web), was on the order of a factor of 5 to 9 (i.e., the ratio of 90th to 10th percentiles of the model predictions for PCB concentrations in piscivorous fish). Uncertainty arises from a number of causes, but especially because models are only simplified approximations of the ecosystem. In the case of food chain models, the descriptions of the various aspects of the bioaccumulation process also rely upon many empirical relationships and correlations, all of which contain potential errors.

Applying a food chain model correctly (that is to say, making reliable predictions that are free of preventable errors) is an involved process. To correctly apply a model, the investigator must have an adequate understanding of the model and the science upon which it is based. This understanding may be gained from training courses [such as those offered by the EPA Center for Exposure Assessment Modeling (CEAM), the Manhattan College Summer Institute for Water Pollution Control, or short courses offered in conjunction with scientific conferences such as the annual meetings of the Society of Environmental Toxicology and Chemistry (SETAC)] or "User's Guide" documentation, although these sources may assume the modeler has a fairly advanced background. The modeler should become thoroughly familiar with the data requirements, assumptions and limitations of a model. Much can be learned by reviewing publications and reports documenting prior applications of the model. The modeler must then acquire the site-specific data or validated estimates for all model inputs, run the model, and verify and confirm the predictions (USEPA, 2003b). The goal of confirmation is to determine and quantify the agreement between model predictions and observations. In the case of food web bioaccumulation models, comparisons between predictions and observations should be made for chemical concentrations in the organism or tissue[1] of interest, as well as chemical concentrations

[1]The Gobas, Thomann, and most other food chain models predict chemical concentrations on a whole organism basis. Chemical concentrations in specific tissues can be recomputed from concentrations predicted in the whole organism using (1) measured ratios of chemical concentrations between tissue and

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in organisms at lower trophic levels in the food web (if available). If data for chemical concentrations is available for multiple trophic levels in the food web at the site, it may be appropriate to confirm the model predictions in terms of the BMFs that can be calculated from these data. Other ways to evaluate or test the performance of a model and the robustness of its predictions include peer reviews, numbers of past applications and their successes, and similarity of other applications to the chemical, food web, and site of interest. The following references are offered as resources to investigators considering the use of food chain models to predict BMFs: Gobas (1993); Gobas et. al. (1998); Arnot and Gobas (2004); Thomann (1989); Chapra (1997); Campfens and Mackay (1997); Morrison et al. (1996); Thomann et al. (1992); Connolly, (1991); Barber et al. (1991); Thomann and Meuller (1987); Thomann and Connolly (1984); and, Connolly et al. (1992). The investigator should also be aware that EPA has developed quality assurance guidance applicable to model applications, which outlines the elements of a QAPP for modeling (USEPA, 2002).

Food chain models with the characteristics and desirable qualities summarized above include the models of Gobas (1993) and Thomann et al. (1992). These two models are widely accepted in the scientific community and are being used in a number of scientific and regulatory applications. Many other models are available, as discussed in USEPA (2003). Since some of these latter models have extensive input data requirements, and are designed for temporally and spatially variable solutions for the food web, they were not considered to be appropriate for this application. Burkhard (1998) performed a thorough evaluation of the Gobas (1993) and Thomann et al. (1992) steady-state food web models for predicting chemical concentrations in aquatic food webs. This evaluation included assessments of (1) the accuracy and precision of the models, (2) the sensitivity of the predicted concentrations to changes in input parameters, and (3) the uncertainty associated with the concentrations predicted by the models. Burkhard's (1998) evaluation using field data from Lake Ontario (Oliver and Niimi, 1988) demonstrated that the Gobas and Thomann models have similar predictive abilities for fish species at each trophic level and for chemicals with log K_{ow}s ranging from 3 to 8.

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whole organisms (e.g., Niimi and Oliver, 1989) or (2) the ratio between tissue and whole organism lipid content.

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EPA used the Gobas food chain model[2] to predict FCMs in the National BAF Methodology. The rationale for this choice was discussed in USEPA (2003). In part, EPA selected the Gobas model because the computer program was widely available on the Internet (http://www.rem.sfu.ca/toxicology/models/AQUAWEBv1.2_BIOv1.2.xls). In applying the Gobas model, however, EPA did not use the model's method of accounting for chemical bioavailability. Gobas's method for determining the freely dissolved (bioavailable) concentration of the chemical in water makes no distinction between POC and DOC phases, but rather treats these two phases as one. This is significantly different than the procedure used by EPA in the 2000 Human Health Methodology for determining the concentration of chemical that is freely dissolved in the ambient water, C_w^{fd}. To compensate for this discrepancy in the methods of accounting for bioavailability, EPA sets the concentration of the TOC in the Gobas model to an infinitesimally small value (i.e., 1×10^{-30}). By doing so, the total concentration of the chemical input to the model becomes essentially equal to the C_w^{fd}, due to the negligibly small bioavailability correction.

For the National BAF Methodology, FCMs were determined with the Gobas model using the Lake Ontario food web structure presented in Table 5-4 and the environmental parameters and conditions listed in Table 5-5. For each value of K_{ow} inputted to the Gobas model, predicted baseline BAFs were reported by the model for each organism in the food web. FCMs were calculated from the predicted BAFs using the following equation:

$$FCM_i = \frac{\text{Baseline BAF}_i}{K_{ow}} \qquad \text{(Equation 5-14)}$$

[2] The Gobas food chain model is also known as Aquaweb; the current version is Aquaweb v1.2.

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Table 5-4. Food Web Structure for National BAF Methodology (Flint, 1986; Gobas, 1993)

Species	Trophic Level	Lipid Content	Weight	Diet
Phytoplankton	**1**	0.5%		
Zooplankton (mysids [*Mysis relicta*])	**2**	5.0%	100 mg	Phytoplankton
Benthic Invertebrates (*Diporeia*)	**2**	3.0%	12 mg	Sediment/Detritus
Sculpin (*Cottus cognatus*)	**3**	8.0%	5.4 g	18% zooplankton, 82% *Diporeia*
Alewife (*Alosa pseudoharengus*)	**3**	7.0%	32 g	60% zooplankton, 40% *Diporeia*
Smelt (*Osmerus mordax*)	**3-4**	4.0%	16 g	54% zooplankton, 21% *Diporeia*, 25% sculpin
Salmonids (*Salvelinus namaycush, Oncorhynchus mykiss, Oncorhynchus velinus namaycush*)	**4**	11%	2,410 g	10% sculpin, 50% alewife, 40% smelt

Table 5-5. Environmental Parameters and Conditions Used for Determining FCMs for the National BAF Methodology

Parameter	Value
Mean water temperature	8C
Organic carbon content of the sediment	2.7%
Metabolic transformation rate constants (all organisms)	0.0 d^{-1}
$_{socw}/K_{ow}$	23

Using Equation 5-14, FCMs were calculated for each trophic level in the Lake Ontario food web. Table 5-6 lists the FCMs for trophic level 2 (zooplankton), trophic level 3 (forage fish), and trophic level 4 (piscivorous fish). The FCMs determined for the national BAF methodology for trophic levels 2 through 4 are also plotted as a function of the $\log K_{ow}$ of the

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chemical in Figure 5-9. As shown by the relationships between FCMs and $\log K_{ow}$s in Table 5-6 and Figure 5-9, significant biomagnification at trophic levels 3 and 4 occurs for nonmetabolized organic chemicals with $\log K_{ow}$s between about 5 and 8.5. The highest FCMs (13.3 for TL3 and 24.7 for TL 4) were determined for nonmetabolized organic chemicals with $\log K_{ow}$s in the range of 6.7 to 7.0. Constant FCMs of 1 (no biomagnification) were determined for trophic level 2.

Table 5-6. Food-Chain Multipliers for Trophic Levels (TLs) 2, 3, and 4 (Mixed Pelagic and Benthic Food Web Structure and $_{socw}/K_{ow}$ = 23)

Log K_{ow}	TL 2	TL 3[a]	TL 4	Log K_{ow}	TL 2	TL 3[a]	TL 4
4.0	1.00	1.23	1.07	**6.6**	1.00	12.9	23.8
4.1	1.00	1.29	1.09	**6.7**	1.00	13.2	24.4
4.2	1.00	1.36	1.13	**6.8**	1.00	13.3	24.7
4.3	1.00	1.45	1.17	**6.9**	1.00	13.3	24.7
4.4	1.00	1.56	1.23	**7.0**	1.00	13.2	24.3
4.5	1.00	1.70	1.32	**7.1**	1.00	13.1	23.6
4.6	1.00	1.87	1.44	**7.2**	1.00	12.8	22.5
4.7	1.00	2.08	1.60	**7.3**	1.00	12.5	21.2
4.8	1.00	2.33	1.82	**7.4**	1.00	12.0	19.5
4.9	1.00	2.64	2.12	**7.5**	1.00	11.5	17.6
5.0	1.00	3.00	2.51	**7.6**	1.00	10.8	15.5
5.1	1.00	3.43	3.02	**7.7**	1.00	10.1	13.3
5.2	1.00	3.93	3.68	**7.8**	1.00	9.31	11.2
5.3	1.00	4.50	4.49	**7.9**	1.00	8.46	9.11
5.4	1.00	5.14	5.48	**8.0**	1.00	7.60	7.23
5.5	1.00	5.85	6.65	**8.1**	1.00	6.73	5.58
5.6	1.00	6.60	8.01	**8.2**	1.00	5.88	4.19
5.7	1.00	7.40	9.54	**8.3**	1.00	5.07	3.07
5.8	1.00	8.21	11.2	**8.4**	1.00	4.33	2.20
5.9	1.00	9.01	13.0	**8.5**	1.00	3.65	1.54
6.0	1.00	9.79	14.9	**8.6**	1.00	3.05	1.06
6.1	1.00	10.5	16.7	**8.7**	1.00	2.52	0.721
6.2	1.00	11.2	18.5	**8.8**	1.00	2.08	0.483
6.3	1.00	11.7	20.1	**8.9**	1.00	1.70	0.320
6.4	1.00	12.2	21.6	**9.0**	1.00	1.38	0.210
6.5	1.00	12.6	22.8				

[a] The FCMs for trophic level 3 are the geometric mean of the FCMs for sculpin and alewife.

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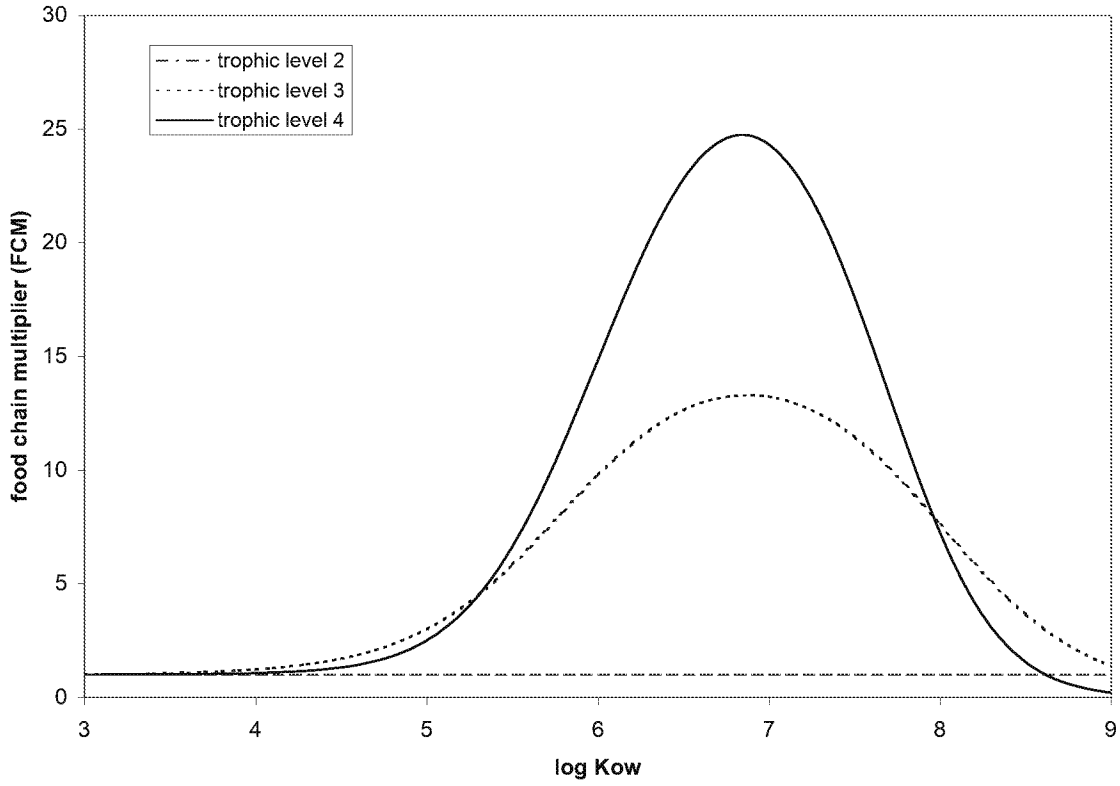

FIGURE 5-9. The FCMs determined for the national BAF methodology for trophic levels 2 through 4.

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5.2.2.3 Site-specific adjustment of food chain model parameters

As noted previously, conditions and parameters of relevance to a food chain model at a particular site may be different from the representative values used in the national methodology calculations. If the investigator determines that site conditions and parameters differ significantly from the nationally-representative conditions, it may be appropriate to recompute FCMs using site-specific conditions as input to the food chain model. A number of food chain model parameters can be adjusted to improve predictions of biomagnification at the site of interest. The most important of these are related to factors which primarily determine bioaccumulation of nonionic organic chemicals by fish (Burkhard et al. 2003a). These include:

- chemical disequilibrium between sediment and water (i.e., $_{socw}/K_{ow}$ and sediment organic carbon content);
- the relative benthic/pelagic connectivity of the food web;
- the length of the food chain (i.e., the trophic level of the organism); and
- species-specific parameters for organisms in the food chain/web (lipid content and weight), as well as bioenergetic parameters (e.g., growth, respiration, consumption) which are computed as allometric functions of organism weight and water temperature in the Gobas and Thomann models.

The sensitivity of model-predicted FCMs to these factors is discussed in Burkhard (1998) and Burkhard et al. (2003b). In all cases, parameter adjustment should be limited to values determined to be representative and unbiased based upon data for the site, species, and chemical of interest. EPA does *not* consider site-specific adjustment of parameters associated with the other two factors - the hydrophobicity of the chemical (K_{ow}) and the rate of chemical metabolism in the food chain - to be appropriate since these parameters are properties of the chemical and (in the case of metabolism rate) the food chain organisms.

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5.2.2.4 Selection of a food web structure

To determine FCMs with a food web model such as the Gobas model, the food web structure must be defined. Food web structures vary across ecosystems and for different organisms within the ecosystem, and these differences also influence bioaccumulation (Burkhard et al. 2003(b)). For highly-hydrophobic chemicals (logK_{ow}s of 6 to 7), the food web structure becomes a very significant factor in bioaccumulation predictions. The information necessary to construct a food web includes the diet of the individual organisms composing the food web and their weights and lipid contents. Based upon Burkhard's (1998) sensitivity analysis, model predictions made by the Gobas model were relatively insensitive to organism weights and feeding preferences of piscivorous fish for all K_{ow}s. For chemicals with higher log K_{ow}s, the predictions were more sensitive for $_{socw}$, feeding preferences of forage fish upon benthic invertebrates, and lipid contents. The most sensitive input parameter was the feeding preferences of forage fish, that is, the percentage of zooplankton (pelagic component) and benthic invertebrates (benthic component) in their diet. The benthic/pelagic composition of the food web is, EPA believes, the most important characteristic for defining the structure of the food web for piscivorous fish because transfer of chemicals from the sediment to piscivorous fish occurs almost exclusively via their diet.

Food webs differ widely in their benthic/pelagic compositions among ecosystems, among individual species, and among different age classes of species within an ecosystem. Of all the ecosystem types, the purely pelagic food webs might be the least common for piscivorous fish. However, purely pelagic food webs have been found in remote Ontario lakes for lake trout (Rasmussen et al. 1990) and in Adirondack lakes for brook trout and yellow perch (Havens, 1992). Purely benthic food webs are more common than purely pelagic food webs, but are still rather limited in nature. Some examples of purely benthic food webs can be found in tidal and estuarine ecosystems, such as the food webs for flounder in New Bedford harbor (Connolly, 1991) and striped bass in the tidal Passaic River (Iannuzzi et al. 1996). Mixed food webs are common in all ecosystems and, EPA believes, far outnumber the purely pelagic and benthic food webs. There are numerous examples of mixed benthic/pelagic food webs, such as the food webs for lake trout in the Great Lakes (Flint, 1986; Morrison et al. 1997), lobster in the New Bedford

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harbor (Connolly, 1991), whitefish and rainbow trout in the Fraser River (Gobas et al. 1998), white perch in the Chesapeake Bay (Baird and Ulanowicz, 1989), and perch, bass, and crappie in Little Rock Lake (Martinez, 1991). Purely pelagic and/or benthic species can exist in ecosystems containing species with a mixed benthic/pelagic food web, for example, flounder and lobster in New Bedford harbor (Connolly, 1991).

5.2.2.5 Alternative food chain models

Food chain models of chemical bioaccumulation are continually being developed and refined, so in the future, EPA may consider the use of other appropriately validated food web models for the derivation of FCMs. Any model considered should have the characteristics and qualities outlined in Section 5.2.2.2. and would have to be subjected to a validation process to address the issues of (1) accuracy and precision of the model predictions, (2) input parameter sensitivities, and (3) uncertainties associated with the model predictions.

5.2.3 Predicting Site-Specific Baseline BAFs using K_{ow} and Food Chain Multipliers (Method 4b)

A site-specific baseline BAF for nonionic organic chemicals can also be predicted using the product of the chemical's K_{ow} and a FCM for a particular trophic level under site-specific conditions. Method 4 uses the following baseline BAF equation:

$$\text{Baseline BAF}_i = K_{ow} \cdot FCM_i \qquad \text{(Equation 5-15)}$$

where:

FCM_i = the food-chain multiplier for trophic level *i*, determined from appropriate field data or predicted for site-specific conditions

K_{ow} = *n*-octanol-water partition coefficient

The K_{ow} can be substituted for the BCF when predicting a site-specific baseline BAF for hydrophobic organic chemicals, particularly for those chemicals that are poorly metabolized by aquatic organisms, because the K_{ow} is strongly correlated with the BCF for these chemicals. As

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with Method 4a, the K_{ow} must be adjusted with a FCM to account for chemical biomagnification through the food web as a result of dietary exposures. Method 4b is appropriate for non- or poorly-metabolized nonionic organic chemicals, but can also be applied to certain ionic chemicals having similar partitioning behavior. Method 4b is most appropriate for nonionic organic chemicals with log K_{ow}s greater than or equal to 4 and low rates of metabolism. This approach may overpredict BAFs for chemicals that are metabolized by aquatic organisms, because metabolism is not incorporated in either the K_{ow} or the FCM. Because the K_{ow} is assumed to be equal to the baseline BCF, the organic carbon and lipid normalization procedures used in Method 4a (equation 5-7) are not needed here. The determination of appropriate FCMs and selection of K_{ow} values are discussed below; further details on these topics can be found in Section 4.4 and Appendix B of TSD Volume 2 (USEPA, 2003).

Calculating a site-specific BAF using Method 4b is presented in the following example.

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Site-Specific BAF Predicted Using the Product of the Chemical K_{ow} and a FCM (Method 4b)

This example illustrates the prediction of a site-specific BAF using Method 4b, again using hydrophobic nonionic chemical k. To predict a site-specific BAF using Method 4b, the investigator uses the product of the K_{ow} for the chemical and a FCM, which must be determined for the chemical, site, and trophic level of the target organism. In this method, K_{ow} is assumed to be equal to the baseline BCF, as discussed in Section 5.2.3. Method 4 requires selection of an appropriate K_{ow} for the chemical, which is multiplied by an appropriate FCM to account for biomagnification.

For this example, Method 4b will be used to predict a site-specific BAF for crayfish using the same food chain structure presented in the Method 4a (page 5-37) example, based on the following data from the site:

MEASUREMENT	AVERAGE VALUE
Chemical k Total Water Concentration	0.20 ng/L
Chemical k Sediment Concentration	1.95 g/kg
Water Column POC	0.54 mg/L
Water Column DOC	3.5 mg/L
Sediment Organic Carbon	7.4%
Phytoplankton Lipid	1.2%
Zebra Mussel Lipid	1.3%
Crayfish Lipid	1.7%

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Site-Specific BAF Predicted Using the Product of the Chemical K_{ow} and a FCM (Method 4b continued)

Determining the chemical K_{ow}

Guidance for selecting an appropriate K_{ow} for the chemical is provided in Section 5.2.3.2 and TSD Volume 2 (USEPA, 2003). For the purposes of this example, a K_{ow} value of 2×10^4 (log K_{ow}= 4.3) will again be used for chemical k.

Calculating the Freely Dissolved Chemical Concentration and Sediment-Water Fugacity Gradient

The freely dissolved chemical concentration (C_w^{fd}) and the sediment-water fugacity gradient ($_{socw}/K_{ow}$) should be calculated from the site data. The freely dissolved chemical concentration can be calculated from the total chemical concentration and the freely dissolved fraction calculated using equation 3-6:

$$f_{fd} = \frac{1}{1 + \frac{0.54mg - POC}{L} \cdot 2.0\times10^4 \frac{L}{kg} \cdot \frac{kg}{10^6 mg} + 0.08 \cdot \frac{3.5mg - DOC}{L} \cdot 2.0\times10^4 \frac{L}{kg} \cdot \frac{kg}{10^6 mg}} = 0.984$$

$$C_w^{fd} = f_{fd} \cdot C_w = 0.984 \cdot (0.20\ ng/L) = 0.197\ ng/L$$

The sediment-water fugacity gradient can then be calculated using equation 4-3:

$$\Pi_{SOCW} = \frac{C_{SOC}}{C_w^{fd}} = \frac{\left(\frac{1.95\mu g}{kg} \cdot \frac{kg}{0.074kg - SOC}\right)}{0.197ng/L} \cdot \frac{1000ng}{\mu g} = 1.34\times10^5 L/kg - SOC$$

$$\frac{\Pi_{SOCW}}{K_{ow}} = \frac{1.34\times10^5 L}{kg - SOC} \cdot \frac{kg}{2.0\times10^4 L} = 6.71$$

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Site-Specific BAF Predicted Using the Product of the Chemical K_{ow} and a FCM (Method 4b continued)

The Gobas Aquaweb model can be downloaded from http://www.rem.sfu.ca/toxicology/models/AQUAWEBv1.2_BIOv1.2.xls as an Excel spreadsheet, which is simple to apply. For this example, several inputs to the spreadsheet must be modified. On the EP (environmental parameters) sheet, the organic content of water must be changed to 1.0×10^{-30} kg/L (this was discussed on page 5-52) and the organic carbon content of sediment must be changed to 7.4%, the site-specific value. On the CP (chemical specific parameters) sheet, data for chemical k must be entered. These include the log K_{ow} of 4.30, the total water concentration of 0.197 ng/L (the freely dissolved concentration), and the sediment concentration of 1.95 ng/g.

Several changes are also required on the BP (biological parameters) sheet, although the Aquaweb model includes each of the foodweb species of interest for this site. Zebra mussels are the first invertebrate species in the model. The lipid content of zebra mussels should be 1.3%, and the feeding preferences (fraction of diet) should be changed to 0.75 phytoplankton and 0.25 sediment/detritus. Crayfish are the fifth invertebrate species in the model. The lipid content of crayfish should be 1.7%, and the feeding preferences (fraction of diet) should be changed to 1/3 phytoplankton and 2/3 invertebrate 1 (zebra mussels). For both invertebrate spesies, we will accept the default 5% pore water ventilation fraction, although the spreadsheet notes that site-specific data should be considered.

Once these data are input, the model predicts a concentration of chemical k in crayfish of 0.199 ng/g-wet. The site-specific total BAF for chemical k in crayfish can be calculated from Equation 3-1:

$$\text{Total BAF} = \text{BAF}^{t}_{i,T} = \frac{C_t}{C_w} = \frac{0.199\ ng/g}{0.20\ ng/L}\cdot\frac{1000g}{kg} = 995\ L/kg$$

The baseline BAF for chemical k in crayfish can be calculated from the total BAF using Equation 3-4, recalling that the freely dissolved fraction of chemical k in the site water column was 0.984:

$$\text{Baseline BAF}_i = \left[\frac{\text{BAF}^{t}_{i,T}}{f_{fd}} - 1\right]\cdot\frac{1}{f_l} = \left[\frac{995L/kg}{0.984} - 1\right]\cdot\frac{g-wet}{0.017g-lipid} = 5.94\times10^4\ L/kg-l$$

The FCM for chemical k in crayfish at this site can also be calculated, using equation 5-14:

$$\text{FCM} = \frac{\text{Baseline BAF}}{K_{ow}} = \frac{5.94\times10^4\ L}{kg-l}\cdot\frac{kg-l}{2.0\times10^4\ L} = 2.98$$

A number of assumptions are associated with predicting site-specific baseline BAFs by Method 4b. First, it is assumed that the K_{ow} is equal to the chemical's baseline BCF, an assumption that is only valid for non-metabolized chemicals. Second, it is assumed that there is no metabolism of the chemical in the food web. Third, the other assumptions incorporated into the FCMs (whether measured or modeled) are directly incorporated into the predictions made with Method 4b. For detailed information on the assumptions incorporated into the FCMs, refer to Section 5.2.2.

Method 4b assumes that the K_{ow} is equal to the chemical's baseline BCF. This assumption is supported by equilibrium partitioning theory. This theory assumes that (1) the bioconcentration process can be viewed as a partitioning of a chemical between the lipid of aquatic organisms and water and the K_{ow} is a useful surrogate for this partitioning process, and (2) a linear relationship exists between the K_{ow} and the BCF. Mackay (1982) demonstrated the usefulness of K_{ow} as a surrogate for this partitioning process by presenting a thermodynamic basis for the partitioning process for bioconcentration. In theory, it follows that the baseline BCF (i.e., BCF based on the concentration of chemical in lipid of organisms and freely dissolved in water) for organic chemicals should be similar, if not equal to, the K_{ow}. This theory is supported by a considerable body of empirical data. As summarized by Isnard and Lambert (1988), numerous studies have demonstrated a linear relationship between the log K_{ow} for organic chemicals and the log BCF measured for fish and other aquatic organisms exposed to those chemicals. In addition, when the regression equations are constructed with BCFs reported on a lipid-normalized basis, the slopes and intercepts are not significantly different from 1 and 0, respectively. For example, de Wolf et al. (1992) adjusted a relationship reported by Mackay (1982) to a lipid-normalized basis and obtained the following relationship:

$$\log BCF = 1.00 \log K_{ow} + 0.08 \qquad \text{(Equation 5-16)}$$

For highly-hydrophobic chemicals (log K_{ow} >6.0), reported BCFs are often not equal to the K_{ow} even for nonmetabolized chemicals, because the measurements were not performed and/or reported with appropriate experimental conditions. BCFs for nonmetabolized chemicals *are* equal to the K_{ow} when the BCF values meet the following quality assurance criteria:

ED_006490_00009970-00318

- reported on a lipid-normalized basis,
- determined using the concentration of the chemical that is freely dissolved in the exposure water,
- corrected for growth dilution,
- determined under steady-state conditions or from accurate measurements of the chemical's uptake (k_1) and elimination (k_2) rate constants, and
- determined with no solvent carriers in the exposure water.

5.2.3.1 Validation of Method 4b

As noted in Section 4.6.1, Burkhard et al. (2003b) have validated and compared the predictive powers of Methods 2 (i.e., baseline BAFs predicted from field-measured BSAFs) and 4b. The validation exercises were performed using data collected from a number of diverse aquatic ecosystems: Lake Ontario, Green Bay/Fox River, the Hudson River, and Bayou d'Inde, Louisiana. With these data sets, baseline BAFs predicted using Method 4b were plotted against field-measured baseline BAF (i.e., Method 1) values. The agreement between baseline BAFs predicted using Method 4b and Method 1 baseline BAF values is generally good for Green Bay, although not as good as the agreement between Method 2 and Method 1 baseline BAFs (Burkhard et al. 2003b). In Green Bay, 59% of the baseline BAFs predicted using Method 4b were within a factor of 2, and 93% were within a factor of 5, of the measured baseline BAFs (Table 5-7). The validation exercises using the Green Bay/Fox River and Hudson River data are described in detail in Burkhard et al. (2003b). Figure 4-2 compares Method 2 and Method 4b predictions to the baseline BAFs measured in the Green Bay and Hudson River ecosystems.

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Table 5-7. Validation Statistics for Method 4b: Ratio Between Predicted and Measured Baseline BAFs (Baseline $BAF_{predicted}$/Baseline $BAF_{measured}$) based on PCB concentration data from Green Bay, Lake Michigan and the Hudson River.

Location	Method 4b: Exceedance Levels and Comparison Statistics					
	95%	Mean	Median	5%	% within 2x	% within 5x
Green Bay						
Zone 1	0.32	1.17	0.89	2.75	69.8	98.1
Zone 2a	0.17	1.17	0.74	3.40	54.6	91
Zone 2b	0.23	1.18	0.83	3.01	61.0	94.9
Zone 3a	0.33	1.58	1.05	4.71	64.0	94.7
Zone 3b	0.23	1.35	0.90	4.15	60.5	94
Zone 4	0.15	1.43	0.61	5.28	40.5	82.2
All Zones	0.21	1.30	0.84	3.90	58.6	92.7
Hudson River						
RM 194	0.06	0.16	0.11	0.38	3.6	25.3
RM 189	0.12	0.26	0.20	0.55	9.0	55
RM 169	0.10	0.95	0.41	1.89	35.3	76.5
RM 144	0.42	0.72	0.67	1.14	76.5	100
RM 122	0.40	0.70	0.67	1.27	80.0	100
RM 114	0.4	0.78	0.73	1.29	76.9	100
All Stations	0.08	0.50	0.24	1.07	26.3	60.7

RM = river mile

The accuracy of baseline BAFs predicted with Method 4b in the Hudson River varied among sites. Generally, the predicted baseline BAFs are biased low; this is evident in Table 5-8, where the mean and median predicted/measured ratios are less than 1 for all locations. At three of the six stations in the Hudson River (river miles (RM) 114, 122, and 144), there was good agreement between predicted and measured baseline BAFs (>75% within a factor of 2, and 100% within a factor of 5; Table 5-7). However, for river mile 169, agreement was not as good (35% within a factor of 2; 76% within a factor of 5). Finally, at two sites (river miles 189 and 194), there was substantial underprediction of measured baseline BAFs with Method 4b. On the other hand, for the Hudson River data set, the variability associated with baseline BAFs

ED_006490_00009970-00320

predicted using Method 4b was generally smaller than that associated with Method 2. Burkhard et al. (2003b) discuss several factors that might be involved with the underprediction of the baseline BAFs for river miles 169, 189, and 194 using Method 4b. These include (1) the use of FCMs (Table 5-6) derived using conditions and parameters for the nation instead of for the Hudson River, (2) the use of field samples that were not temporally and/or spatially coordinated and/or representative of the ecosystem, and (3) the sampling of an ecosystem with rapidly changing conditions in recent history due to unusual conditions in the river.

Table 5-8. Summary Statistics: Differences Between Log Baseline BAFs Predicted with Method 4b and Log Baseline BAFs Measured from Lake Ontario (Oliver and Niimi, 1988) for Chemicals with Log K_{ow} Exceeding 4

Statistic	Organism				
	Sculpin	Alewife	Small Smelt	Large Smelt	Piscivorous Fish
Average	0.01	0.04	0.09	0.28	0.08
Standard Deviation	0.35	0.36	0.37	0.35	0.36
Count	51	49	46	47	57
Median	0.02	0.06	0.14	0.30	0.08
Within 2x	63%	59%	61%	47%	58%
Within 5x	94%	94%	94%	92%	96%
Negative Residual	53%	53%	59%	72%	56%
Positive Residual	47%	47%	41%	28%	44%

Burkhard et al. (1997) also evaluated the predictiveness of Method 4b against field-measured baseline BAFs for trophic level 3 fish sampled from the Bayou d'Inde for selected chlorinated benzenes, chlorinated butadienes, and hexachloroethane. Bayou d'Inde is a lowland channel that meanders through a brackish-freshwater marsh that is influenced by tide. This ecosystem is very different from either the Great Lakes or the Hudson River and provides a useful demonstration of the applicability of Method 4b across different ecosystems. Because this evaluation of Method 4b was conducted before the development of the final National BAF

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Methodology, it was performed with FCMs and default values for POC and DOC that are marginally different from those that are used in the National BAF Methodology (USEPA, 2003). Burkhard et al. (1997) found good agreement between the predicted and measured baseline BAFs for both the fish and invertebrates sampled. Overall, approximately 90% of the Method 4b-predicted baseline BAFs were within a factor of 5 of the measured baseline BAFs, and the median ratio of the predicted baseline BAFs to the measured baseline BAFs was 1.64.

The EPA also compared the baseline BAFs predicted with Method 4b to measured BAFs for the Lake Ontario ecosystem (Table 5-8). The average differences between measured and predicted baseline BAFs were small for both forage and piscivorous fish, and more than 90% of the baseline BAFs predicted with Method 4b were within a factor of 5 of the measured BAFs. The residuals (i.e., the differences between predicted and measured BAFs) were evenly distributed, except for the large smelt. The trophic level for the large smelt is estimated to be 3.5, owing to its consumption of smaller forage fish, and consequently, it was anticipated that the predicted baseline BAFs with trophic level 3 FCMs would be slightly lower than the measured BAFs for this species.

As summarized above, the predictive accuracy of Method 4b has been evaluated with field data from four different ecosystems. For the Lake Ontario, Green Bay/Fox River, and Bayou d'Inde ecosystems, baseline BAFs predicted with Method 4b were in excellent agreement with the measured BAFs: More than 90% of the predicted baseline BAFs were within a factor of 5 of the measured baseline BAFs. In the Hudson River, for three of the sampling stations, baseline BAFs predicted with Method 4b were in excellent agreement with measured BAFs: 100% of the predictions were within a factor of 5 of the measured baseline BAFs. For the other three sampling stations in the Hudson River, baseline BAFs predicted with Method 4b were much smaller than the measured BAFs, but the predictions were consistent with those based on a complex site-specific, time-dependent food web bioaccumulation model (QEA, 1999).

Overall, EPA believes that Method 4b provides excellent predictions for ecosystems that have not recently experienced a major change or disruption in chemical loadings or flows. Of all the ecosystems examined, the extreme temporal dynamics observed for several important factors

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(e.g., fish lipid content, food web structure, exposure concentrations) in the Hudson River makes this site a severe test of all the BAF methodologies. In fact, the Hudson River data set may arguably fail to meet the sampling and data quality considerations specified in Section 3.4 and 3.5 for deriving baseline BAFs from field data. Nonetheless, EPA believes that the application of the BAF methods to this location was a useful exercise and illustrates that useful predictions are possible using Method 4b in ecosystems with extreme temporal dynamics.

5.2.3.2 Selection of appropriate K_{ow}s for partitioning (bioavailability) predictions

The K_{ow} of the chemical of interest is used in several components of the BAF methodology, for example, to estimate BAFs, hydrophobicity, and partitioning in water; as well as in the prediction of baseline BAFs from BSAFs (Method 2) and in the use of the food chain model to predict FCMs for Method 4. Each of these procedures is highly sensitive to the value selected for K_{ow}. Thus, it is important for the investigator to search the literature and chemical property databases for all available data for K_{ow} of the chemical of interest, and then select the most accurate and appropriate value of K_{ow} for the chemical. Although a variety of methods are available to measure or estimate[3] K_{ow} values, the reliability of these methods varies according to the K_{ow} of the chemical. In addition, many unreliable or erroneous K_{ow} values can be found in the literature or in chemical property databases (Linkov et al. 2005; Pontolillo and Eganhouse, 2002).

A detailed approach for selecting reliable K_{ow} values was published in Appendix B of TSD Volume 2 (USEPA, 2003). EPA's methodology for selecting K_{ow} values divides the range of K_{ow}s into three groups ($\log K_{ow} < 6$, $6 < \log K_{ow} < 8$, and $\log K_{ow} > 8$) to reflect the differences in chemical properties and behaviors due to differing hydrophobicities. In general, "high quality" measured values (i.e., data judged to be reliable based on EPA guidelines) are preferred over estimates. K_{ow}s measured by the slow stir method are considered reliable up to a value of 10^8. Shake flask K_{ow} measurements are reliable up to 10^6 as long as sufficient attention is given to

[3] For example, the EPI (Estimation Programs Interface) suite of physical/chemical property and environmental fate estimation models developed by the EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm) includes KOWWIN. This model estimates the log K_{ow} of chemicals using an atom/fragment contribution method.

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micro emulsion effects; for classes of chemicals that are not highly sensitive to emulsion effects (i.e., polycyclic aromatic hydrocarbons) this range may extend to $10^{6.5}$. For chemicals with log $K_{ow} > 5$, it is highly unlikely to find multiple "high quality" measurements. Therefore, assigning K_{ow}'s from estimation techniques may be necessary. When multiple K_{ow} values are found, evaluating the quality of the data (measured or estimated) should include checking the consistency between the values. What is considered reasonable agreement in log K_{ow} data depends primarily on the magnitude of the log K_{ow} value. Therefore, EPA has established the following ranges of acceptable variation for this exercise:

- 0.5 for log $K_{ow} > 7$,
- 0.4 for 6 log K_{ow} 7 , and
- 0.3 for log $K_{ow} < 6$.

Statistical methods should be applied to K_{ow} data as appropriate. However, the investigator should recognize that robust estimates are generally difficult to obtain due the paucity of data and the determinate/methodic nature of most measurement error(s).

5.3 RECALCULATING SITE-SPECIFIC BAFS FROM BASELINE OR NATIONAL BAFS

A site-specific BAF for a nonionic organic chemical can be recalculated from a baseline or national BAF by using values for the aquatic organism lipid content and/or the organic carbon (DOC and POC) concentrations that are representative of conditions at the site. This is Method 5 of EPA's site-specific BAF methodology. The investigator can modify one or both of these parameters in the site-specific recalculation of the BAF by:

- conducting site-specific field studies to generate representative data,
- conducting a literature or database search to obtain data more representative of local conditions, or

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- selecting an appropriate subset of the national database that EPA used to derive the default values.

Method 5 is applicable to nonionic organic chemicals, and similarly-behaving ionic organic chemicals. The formula for recalculating a site-specific BAF from the baseline BAF is:

$$\text{Site Specific BAF}_{i,t}^{T} = (\mathrm{f}_{l} \cdot \text{Baseline BAF}_{i} + 1) \cdot \mathrm{f}_{fd} \qquad \text{(Equation 5-17)}$$

where:

f_{fd} = fraction of the total concentration of chemical that is freely dissolved in the water column at the site

f = fraction of the organism tissue that is lipid

Equation 5-17 is a rearrangement of the equation relating the total and the baseline BAF for nonionic organic chemicals (equation 3-3). In other words, *recalculating* a site-specific BAF means converting a baseline BAF into a total BAF using values for the tissue lipid fraction and/or dissolved chemical fraction in water that are most appropriate for the organism and the site.

Site-Specific BAF Method 5

- Recalculating site-specific BAFs from baseline BAFs, with 2 options:
  - 5a. Adjustment for site-specific lipid content, and/or
  - 5b. Adjustment for site-specific DOC

Although EPA uses national default values of lipid fraction to derive national human health AWQC, States and authorized Tribes are encouraged to use local or regional data on the lipid content and consumption rates of consumed aquatic species when adopting criteria into their own water quality standards. The use of such locally or regionally derived (i.e., site-specific) data is encouraged over national-scale data because local or regional consumption

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patterns of fish and shellfish (and thus the amount of lipid consumed from aquatic organisms) can differ from national consumption patterns, and because lipid contents of specific organisms at a site can vary from nationally derived values due to factors and conditions of the ecosystem. Likewise, EPA encourages States and authorized Tribes to use site-specific data on the organic carbon content of applicable waters when adopting criteria into their own water quality standards. EPA encourages the use of appropriate locally or regionally derived values of DOC or POC over nationally derived values because local or regional conditions that affect DOC and POC concentrations can differ substantially from those represented by nationally derived values.

5.3.1 Assumptions and Limitations

Although both theory and empirical evidence support the concept of adjusting BAFs for lipid content and dissolved chemical fractions to facilitate their extrapolation between species and sites, this practice nevertheless involves making a series of assumptions that deserve to be explicitly stated and evaluated. The same assumptions (and justifications) were made by EPA to support the use of baseline BAFs when deriving national values for nonionic organic chemicals in the 2000 Human Health methodology and TSD Volume 2 (USEPA, 2003). The investigator should refer to these documents for further details regarding the scientific basis for the use of baseline BAFs.

The assumptions associated with adjustment of BAFs by lipid normalization can be stated as:

1. For a given species and exposure condition, the total concentration of a nonionic organic chemical in the tissue of an organism at or near steady state varies in direct proportion to the lipid content in the tissue of interest.

2. The degree of proportionality of chemical concentration with lipid content does not depend on the composition of lipids present in tissue.

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The first assumption is generally supported by the empirical evidence and underlying theory that supports many widely used bioaccumulation models. This assumption is also supported by the findings that for organic chemicals that are not metabolized, BCF is strongly correlated with $K_{ow.}$ (e.g., Veith et al. 1979b; Isnard and Lambert, 1988; de Wolf et al. 1992). In determining K_{ow}s, *n*-octanol is considered to be a surrogate for lipid. Chiou (1985) used triolein (glyceryl trioleate) as a surrogate for lipid and also found good agreement between BCFs and triolein/water partition coefficients.

The second assumption pertains to the utility of the total lipid content as a normalizing factor for species and tissues with widely varying lipid fractions and lipid compositions. The process of normalizing BAFs and BCFs on the basis of the total fraction of tissue that is lipid assumes that lipids are a single, uniform compartment. In reality, total lipid content in fish includes different lipid classes, including relatively polar phospholipids, which are common in cell membranes, and generally nonpolar triacylglycerols, which are common in storage lipids (Henderson and Tocher, 1987). The variation in lipid-partitioning behavior of nonionic organic chemicals is thought to be a function of differences in polarity of lipid classes, as fewer chemicals become associated with the more polar "membrane-bound" lipids than storage lipids (Ewald and Larsson, 1994; van Wezel and Opperhuizen, 1995; Randall et al. 1998).

In practical terms, the potential impact that differences in lipid composition might have on chemical partitioning and lipid normalization seems to be most relevant for very lean tissues (e.g., those less than 1%–2% total lipids). This suggestion is based on observations that lean tissues of some fish species contain a much greater proportion of polar phospholipids (24%–65%) than do "fatty" tissues (1.5%–8.7%; Ewald and Larsson, 1994). Similar observations have been made with populations of ribbed mussels, for which Bergen et al. (2001) reported significantly higher fractions of polar lipids in leaner populations compared with fatter populations. Because of the greater polarity of their lipids, very lean tissues are likely to exhibit different chemical/lipid-partitioning behavior than fatty tissues. Bergen et al. (2001) reported stronger correlations between chemical concentrations and mussels with higher total (and nonpolar) lipid content, which led to their suggestion that lipid normalization may work best above some threshold of lipid content. However, the narrow range of lipid content evaluated in

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their study (about a factor of two) and the reliance on total PCB measurements (as opposed to individual congeners) might have limited their ability to identify meaningful trends between chemical concentrations and lipid content.

Differences in lipid composition in tissues of aquatic organisms also relate to a complication associated with methods used to determine lipid content. Specifically, different solvents have been used to extract lipids, which leads to different quantities (and types) of lipid being extracted from the same tissue of aquatic organisms. In a study by Randall et al. (1991), lipid fraction varied by nearly fourfold among four extraction methods but varied twofold or less among two of the more common extraction methods (chloroform-methanol and acetone-hexane). Following up on their previous work, Randall et al. (1998) report that if different solvents are used to extract lipids and PCB congeners, differences among lipid-normalized concentrations can vary more than fivefold, depending on the solvent combination. The relative difference among lipid extraction methods depends not only on the polarity of the solvent but also the lipid content of the tissue. Because lean tissues contain proportionally more polar lipids than fatty tissues, differences in the lipid extraction efficiency for different solvents tend to be greatest for lean tissues (de Boer, 1988; Ewald et al. 1998). This finding led these authors to caution the use of lipid data from lean tissues that have been extracted using strictly nonpolar solvent systems.

The assumptions associated with adjustment of BAFs by dissolved chemical fraction include:

1. Hydrophobic organic chemicals exist in water in three phases: (1) the freely dissolved phase, (2) sorbed to the organic fraction of suspended solids (i.e., particulate organic carbon), and (3) sorbed to dissolved organic matter. This assumption is supported by a wealth of experimental evidence (Hassett and Anderson, 1979; Carter and Suffet, 1982; Landrum et al. 1984; Gschwend and Wu, 1985; McCarthy and Jimenez, 1985a; Eadie et al. 1990, 1992). The total concentration of the chemical in water is the sum of the concentrations of the freely dissolved chemical and the sorbed chemical (Gschwend and Wu, 1985; USEPA, 1993).

2. Chemicals in the freely dissolved phase of the water are in equilibrium with chemical associated with the DOC and POC (including plankton) phases of the water column. The relationship used by EPA to relate the freely dissolved chemical concentration to the concentrations of chemical associated with DOC and POC (equation 3-12) assumes equilibrium among these phases. For a given ecosystem, DOC and POC

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define the partitioning of the chemical among the three phases. Section 4.2.1 of TSD volume 2 provides background information regarding the derivation of this relationship.

3. The concentration of chemical that is freely dissolved is the best measure of the fraction of nonionic organic chemical available for uptake by aquatic organisms, both in sediment porewaters and ambient surface waters (Suffet et al. 1994; DiToro et al. 1991). Sorption of the chemical to DOC and POC reduces chemical bioavailability to aquatic organisms.

By basing the baseline BAFs on C_w^{fd}, EPA does *not* ignore the chemical associated with dissolved organic carbon (DOC) and particulate organic carbon (POC) in the water column. As stated above, the chemical associated with DOC and POC in the water column is assumed to be in equilibrium with the chemical freely dissolved in the water column. Therefore, any additions or removal of chemical from any of the three phases (i.e., freely dissolved chemical, chemical associated with DOC, and chemical associated with POC) will cause a re-equilibration of the chemical among the three phases. Due to the equilibrium conditions among these three phases, the chemical concentration in the water column expressed using any of the three phases, individually or in combination, is indicative of the chemical concentrations in the other water column phases for a given set of ecosystem conditions.

Reduced chemical uptake by aquatic organisms in the presence of DOC has been extensively reported for both ambient waters and waters containing added DOC (Leversee et al. 1983; Landrum et al. 1985; McCarthy and Jimenez, 1985b; McCarthy et al. 1985; Carlberg et al. 1986; Black and McCarthy., 1988; Servos and Muir, 1989; Kukkonen et al. 1989). For example, it has been reported that the percentage reduction in gill uptake efficiency of benzo[a]pyrene and 2,2',5,5'-tetrachlorobiphenyl in rainbow trout is equal to the percentage reduction in freely dissolved chemical concentration in the presence of DOC (Black and McCarthy, 1988). The authors of this study concluded that only the chemical that was freely dissolved in the water was available for uptake by the fish. Similarly, Landrum et al. (1985), McCarthy et al. (1985), and Servos and Muir (1989) reported that chemical uptake rates were reduced when DOC was present and that the concentration of chemical that is freely dissolved in the water column decreases in proportion to the amount of DOC present in the water. These studies clearly support EPA's assumption that chemical bioavailability of nonionic organic chemicals to aquatic

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organisms is reduced in the presence of DOC and POC. Excellent reviews on the science of bioavailability are provided by Hamelink et al. (1994) and Kukkonen (1995).

5.3.2 Validation of Method 5

Baseline BAFs are based upon the freely dissolved chemical concentrations in ambient water and the chemical concentrations in the lipid fraction of the organisms, and these adjustments allow for better extrapolation of BAFs across species and locations by reducing variability in the extrapolation. To evaluate the merits of this approach, two different comparisons were made. The first involved comparing baseline BAFs and BAFs within and across the different sampling zones of Green Bay for individual species. The second evaluation involved the comparison of baseline BAFs and BAFs across species and ecosystems. In each comparison, baseline adjustment of BAFs was found to significantly reduce the variability in BAFs between zones, species and ecosystems.

Bay-wide baseline BAFs and BAFs were calculated using a sample-size weighted average of the BAFs from each of the sampling zones. The variances of the bay wide baseline BAFs and BAFs were calculated (in log-space) by summing the variances of the chemical concentrations in fish and water across all zones and correcting for the covariance of the chemical concentrations in fish and water within zones. Overall, the baseline BAFs had smaller variances than BAFs, and the baseline adjustments of the BAFs reduced their standard deviations by half (Figure 5-10). This figure also shows that the baseline BAFs varied less across the zones than the BAFs, i.e., the baseline BAFs were nearly constant across zones compared to the BAFs. The BAFs tended to increase from zone 1 to 4 (Figure 5-10), and this difference was more pronounced for the more hydrophobic congeners 149 and 180, consistent with equilibrium partitioning theory. The observed trend of increasing BAFs across zones due to increasing bioavailability of dissolved PCBs, caused by declining particulate and dissolved organic carbon across zones, appeared to be removed by baseline adjustment. The decrease in variances for the baseline BAFs (in comparison with those for BAFs) will result in lower variances for site specific BAFs derived using Method 5. Without baseline adjustment, direct extrapolation of the BAFs from Green Bay to another ecosystem would have larger variances and poor predictive

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power. The above comparisons were all within the Green Bay ecosystem, and they demonstrate that corrections for lipid content and freely dissolved chemical concentrations in water reduce variability associated with the baseline BAFs.

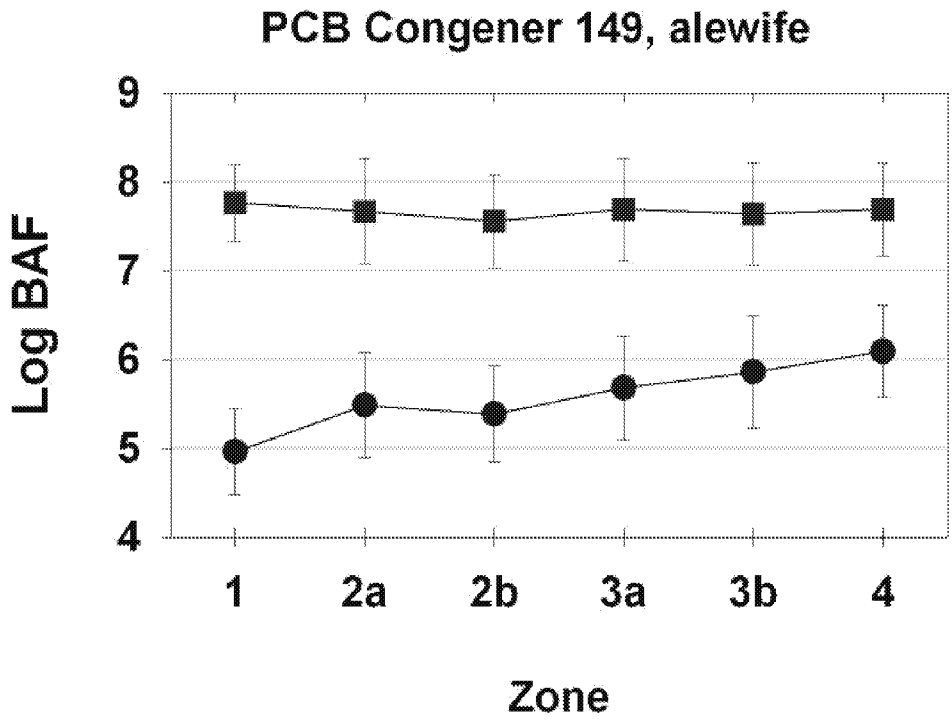

FIGURE 5-10. BAF_T^fs (●) and Baseline BAFs (▪) for PCB congener 149 (2,2',3,4',5',6-hexachlorobiphenyl) (±1 sd) for adult alewife for different spatial zones in Green Bay.

To further evaluate the relative variances associated with BAFs and baseline BAFs, baywide BAFs were compared. Bay-wide BAFs and baseline BAFs were calculated using a sample size weighted average of the BAFs for each of the geographical zones. The variances of the baywide BAFs and baseline BAFs were calculated as described in detail in Burkhard et al. (2003a). The results of these calculations are summarized, by species, using the ratio of 90th to 10th and 95th to 5th percentile exceedance limits in Table 5-9. Overall, the baseline BAFs had smaller ratios than the BAFs and the adjustment/conversion of BAFs to baseline BAFs resulted in an approximately twofold decrease in variability (Burkhard et al. 2003ab).

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Table 5-9. BAFs and Baseline BAFs Confidence Limit Ratios (CLRs) for Adult alewife, Age 4 walleye, and Age 10 carp in Green Bay (All Zones Combined)

PCB Congener	90th to 10th Percentile CLR		95th to 5th Percentile CLR	
	BAF	Baseline BAF	BAF	Baseline BAF
Adult alewife				
18	4.98	3.11	7.86	4.3
52	5.48	2.85	8.90	3.84
149	3.33	1.88	4.70	2.26
180	4.08	2.20	6.10	2.76
Age 4 walleye				
18	3.57	3.50	5.14	5.00
52	4.04	2.74	6.01	3.65
149	3.11	2.12	4.30	2.62
180	3.96	2.12	5.87	2.63
Age 10 carp				
18	4.87	4.23	7.65	6.39
52	6.75	3.49	11.6	4.99
149	5.96	1.87	9.91	2.24
180	7.09	2.17	12.4	2.71

To assess across-ecosystem variabilities, baseline and total BAFs for six PCB congeners (PCBs 22, 52, 85, 118, 146, and 149) were assembled from the Green Bay, Lake Ontario, and Hudson River ecosystems for thirteen fish species (Figure 5-11). When possible, age-class specific BAFs were assembled, and trophic levels for the different species were assigned using nominal/rounded trophic levels. These assignments caused species with slightly lower trophic level positions (e.g., adult gizzard shad with average trophic level of 2.5) to be lumped with species with slightly higher trophic levels (e.g., adult alewife with average trophic level of 3.5) at the nominal trophic levels. The baseline BAFs had substantially lower variability in comparison

to the total BAFs for trophic levels 3 and 4 fishes, i.e., an average 2.3-fold decrease in the coefficients of variation (Figure 5-11). Additionally, the $75^{th}/25^{th}$ and $90^{th}/10^{th}$ percentile ranges were smaller by 2x and 5x, respectively, for the baseline BAFs for both trophic levels. These results demonstrate that the corrections for lipids and freely dissolved chemical concentrations reduce variability when extrapolating BAFs across ecosystems and across species of similar trophic levels. The variability not due to lipid content and freely dissolved chemical concentrations could include: differences in nominal vs. actual trophic level assignments for the individual species; differences in disequilibrium of the ecosystem; analytical and sampling errors and biases; and differences in age, size, growth rate, and/or reproductive status of the individual organisms.

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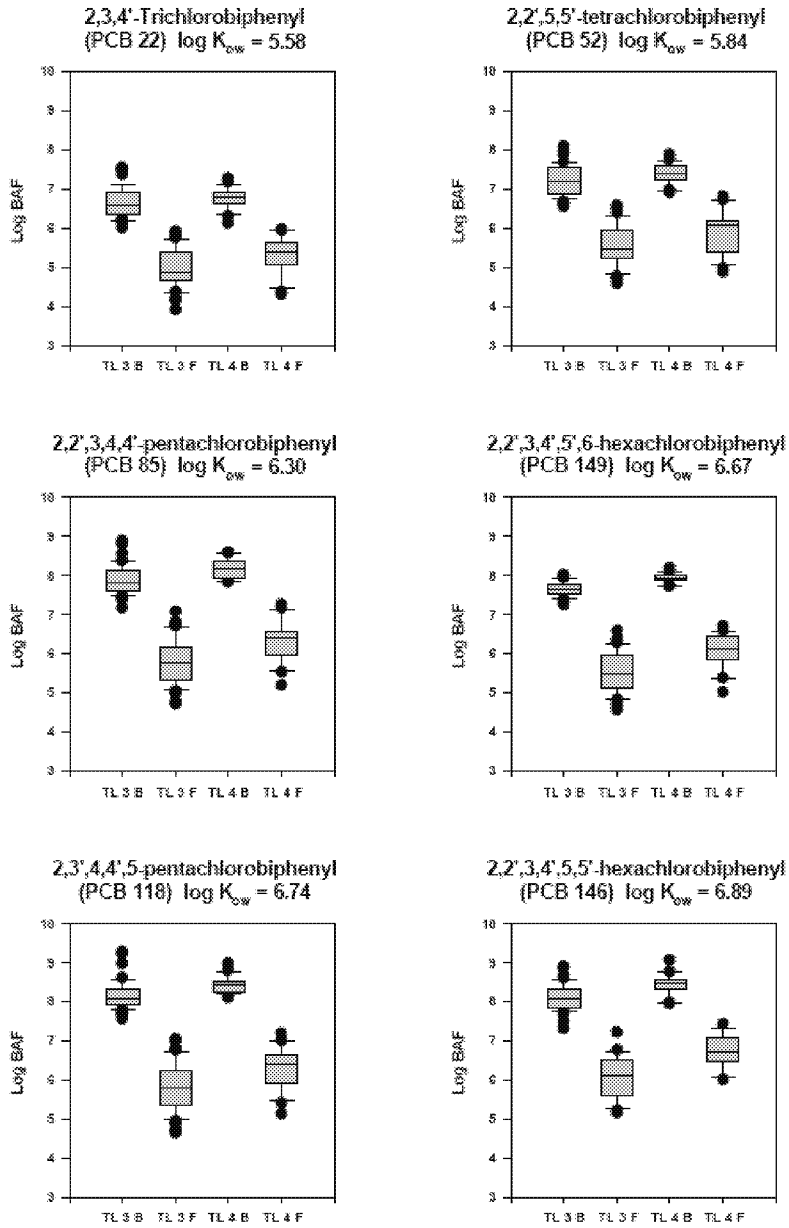

FIGURE 5-11. Box plots comparing baseline (TL 3 or 4 B) and field-measured (TL 3 or 4 F) BAFs for six PCB congeners obtained from Green Bay, Lake Ontario, and Hudson River ecosystems for 13 fish species with samples segregated according to year classes and sampling location, e.g., 4-year-old walleye from zone 4 in Green Bay and adult perch from RM 194 in the Hudson River. For box plots, the median is the line inside the box, the 25th and 75th percentiles are the ends of the box, the 10th and 90th percentiles are the T-lines, and outliers, points beyond the 10th and 90th percentiles, are the dots ( ).

5.3.3 How Can the Lipid Contents of Aquatic Organisms be Determined?

Lipid content is used to adjust BAFs for nonionic organic chemicals because it has been shown to influence the magnitude of bioaccumulation in aquatic organisms (Mackay, 1982; Connolly and Pederson, 1988; Thomann, 1989). Therefore, lipid content in consumed aquatic organisms is an important factor for characterizing potential human exposure to nonionic organic chemicals. Since baseline BAFs are lipid normalized according to the national BAF methodology, recalculating a site-specific BAF (Method 5, equation 5-17) involves multiplying the baseline BAF by the appropriate lipid content. This section discusses how an investigator can determine lipid contents for aquatic organisms and/or tissues consumed from a site, to be used in recalculating a site-specific BAF from a baseline BAF (Method 5).

EPA recommends using local or regional data on the consumption rates and lipid content of consumed aquatic species when recalculating baseline BAFs. The use of such locally or regionally derived (i.e., site-specific) data is encouraged over national-scale data because local or regional consumption patterns of fish and shellfish (and thus the amount of lipid consumed from aquatic organisms) can differ from national consumption patterns. Lipid contents of specific organisms at a site can also vary from nationally derived values due to factors and conditions of the specific ecosystem.

A number of factors can lead to variability in lipid content of aquatic organisms, principally differences in physiology, metabolism, organism health or condition, and feeding ecology among and within species. These factors and, consequently, the lipid content in a particular tissue can vary as a function of season, temperature, reproductive status, migratory patterns, sampling location (both within and across waterbodies), age, size, life stage, the availability of prey, and other factors. For example, the mean percent lipid in fillets of lake trout, *Salvelinus namaycush*, a notoriously "fatty" species, is estimated to be about 12%. This value is about 18 times the mean percent lipid found in fillets of northern pike, *Esox lucius* (0.7%), which illustrates the potentially large variability in lipid content within a single trophic level (both are piscivorous, trophic level-4 fish and are frequently consumed by local populations). Wide

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variation in lipid content can also occur within a species. The coefficient of variation of percent lipid can approach or, in some cases, exceed 100% within a species, even when data are limited to specific tissue types. In addition, the distribution of lipids in a particular aquatic organism is not uniform across all tissue types, resulting in differences in lipid fraction depending on the tissue sampled (e.g., fillet, whole body, muscle). Finally, differences among analytical methods used to extract and measure lipids and associated analytical error can contribute to variability in reported values of lipid fraction.

The following sections offer guidance to the investigator regarding options for determining appropriate lipid contents for site-specific BAF recalculation.

5.3.3.1 Assessing Site-specific Fish Consumption

It is important to identify the fish consumption habits of local populations because the commonly-consumed fish serve as the dietary exposure pathway for bioaccumulative chemicals. The investigator should base their efforts on determining lipid content(s) for the fish species and tissue types that are commonly consumed by the local populations. In all cases, the primary selection criterion should be that the target species is/are among the species commonly consumed in the study area, and that the species is of recreational or sustenance fishing value.

5.3.3.2 Measuring Lipid in Fish

If site-specific lipid data are not otherwise available, the investigator may choose to measure actual lipid contents of the target species and/or tissues. In general, guidance offered in Section 3.4 (*Measuring Chemical Concentrations in Biota*) is applicable for designing a field study (e.g., sample numbers, frequency of collection, location, etc.) to measure lipid content. In this case, however, the investigator should ensure that an appropriate method is used for measuring lipid content. Most methods involve determining lipid content gravimetrically (i.e., by weight), following solvent extraction of the lipid from whole organism or tissue samples. Differences in the polarity of the solvents used to extract lipids from tissue can result in the extraction of different amounts of lipid (Honeycutt et al. 1995). This can lead to variability in

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lipid-normalized concentrations and, consequently, in the site-specific BAF recalculation because of the solvent system used in lipid extraction. Of particular concern are differences in the solvent extraction efficiencies of lipid and chemicals in extremely lean tissues (e.g., <1%-2% lipid). In such tissues, more polar (or mixed polar/nonpolar) solvent systems tend to extract more lipids than do nonpolar solvent systems. This phenomenon is believed to result from the proportionately greater fraction of polar lipids in lean tissues as compared with fatty tissues.

A variety of solvent systems that extract various lipid classes have been proposed for use in normalizing tissue chemical concentrations by lipid content. However, a clear consensus has not emerged on which method is most appropriate for all tissues, species, and nonionic organic chemicals. Although it is desirable to have one standardized lipid extraction method for normalizing concentrations of nonionic organic chemicals, it seems possible that no single method would be equally appropriate for all chemical and tissue types. Different tissues have different lipid compositions that, in turn, may alter the chemical/lipid partitioning process. From a toxicological perspective, the science is presently not clear on which classes of lipids (e.g., phospholipids, free fatty acids, mono-, di-, and triglycerides) are most relevant with respect to different organic chemicals. For example, DDT has been reported to bind to relatively polar membrane-bound lipids, which suggests these membrane lipids might be relevant to DDT toxicity (Chefurka and Gnidec, 1987). Randall et al. (1998) reported that 27% of extractable PCBs were associated with the more polar, membrane–bound lipid pool (i.e., extractable with chloroform/methanol), whereas 73% were associated with the neutral lipid pool (i.e., extractable with hexane). Similarly, de Boer (1988) reported that chlorobiphenyls were associated with both bound (membrane) and unbound (storage) lipid pools in fish. These findings further suggest that membrane-bound lipids should not be ignored when selecting lipid extraction methods.

To promote consistency with other data, including field studies measuring BAFs and BSAFs, EPA recommends the continued use of the Bligh and Dyer (1959) chloroform/methanol extraction method in combination with gravimetric measurement of lipid. The Bligh-Dyer method is recommended because it is widely used for lipid measurements and has been well characterized in terms of the types of lipids extracted. The Bligh-Dyer method also extracts both polar and nonpolar lipids. Based on these and other considerations, Randall et al. (1998) also

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recommend the Bligh-Dyer method as a standard technique for total lipid extraction pending more research to identify the complex neutral chemical/lipid relationships and subsequent development of a definitive standard method. Randall et al. (1998) also recommend that if other lipid extraction methods are used, results should be compared to results obtained using the Bligh-Dyer method to allow conversion of the results to Bligh-Dyer equivalents.

5.3.3.3 Determining Site-Specific Fish Lipid Using a Literature or Database Search

Scientific publications, reports, and online databases also contain data for organism and tissue lipid contents measured in many ecosystems. Determining lipid content in this manner may be an economical and expedient alternative to field measurement, if the appropriate data can be found. The investigator faces two main challenges in doing so, however:

- How to *expediently* find and acquire lipid data for the species, tissue, region, waterbody type, etc. of interest, and
- How to evaluate the quality of the lipid content data.

EPA, other federal and state environmental agencies, and other organizations maintain large electronic databases of aquatic chemistry and ecosystem data that can be accessed via the Internet. These databases are probably the best available resource for lipid content data. Not only are they used as repositories and clearinghouses for aquatic environmental data for many aquatic organisms and ecosystems, but search and retrieval of specific data from these databases is generally straightforward.

The following is an inventory and descriptions of Internet-accessible databases containing lipid content data for many ecosystems, waterbodies and locations in the United States. The databases include:

- Environmental Monitoring and Assessment Program (EMAP)
- National Water Information System (NWIS)
- STORET (STOrage and RETrieval) and Legacy Data Center (LDC)

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- U.S. Army Corps of Engineers BSAF and Lipid Database
- National Study of Chemical Residues in Lake Fish Tissue

Descriptions of each database, and how lipid content data can be accessed from each, are provided in Appendix 5A.

5.3.3.4 How Should Lipid Data be Evaluated?

Lipid content data acquired from the databases described above, or other sources, should be evaluated by the investigator in terms of their usability for establishing lipid contents to be used for recomputing site-specific BAFs. Table 5-10 provides a list of evaluation criteria for lipid data sources. The order of the criteria generally corresponds to their importance in the evaluation process. The three top entries in Table 5-10 *(species of interest, consumed tissue types and method of lipid analysis*) are considered to be essential information. Without these three criteria, lipid content data is essentially unusable because site-specific BAFs are defined in terms of the consumption of specific organisms and/or tissues, and because the extraction method is a critical factor in lipid determination. Care should be taken to review the differences in the extraction method used to measure the lipid content of a given species across studies. As discussed in Section 5.3.2.2, differences in the polarity of solvents used to extract lipids from tissue can result in the extraction of different amounts of lipid. This can lead to variation in lipid contents and, consequently, in recomputed BAFs because of the solvent system used. It may be appropriate to exclude certain data for which differences in lipid contents are believed to be largely due to differences in extraction methods.

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Table 5-10. Evaluation Criteria for Lipid Data Sources

Evaluation Criteria for Lipid Data Sources:
Species of interest
Consumed tissue types for species of interest
Method used for lipid analysis including tissue solvent
QA (% recovery and relative standard deviation)
Collection information (location and time)
Sample factors influencing variability: • Age • Size (length and/or weight) • Sex • Compositing
Presence of under-represented species (e.g., marine fish)
Data quantity
Occurrence of extreme values

The next three criteria in Table 5-10 *(QA statistics, collection location/time, and sample factors influencing variability*) are important because they provide information the investigator can use to understand the sources of variability in lipid data for the target species/tissues. Although the databases often contain considerable data for species of importance for commercial, sport, or sustenance fishing, the *presence of under-represented species* is included in the table because they contain few if any measurements for species that are not. *Data quantity* and *occurrence of extreme values* are useful criteria for evaluating the representativeness of lipid content data.

5.3.3.5 Determining fish lipid using the national default lipid data base

The investigator may also obtain lipid content data by selecting values from the database for fish lipid developed by EPA during the development of the National BAF TSD Volume 2 (USEPA; 2003). Information on the lipid fraction of aquatic organisms was obtained for the

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national database from a variety of primary and secondary sources. The following major sources of lipid data were used in the derivation of national default values of lipid fraction:

- EPA's National Sediment Quality Survey database (USEPA, 2001a)
- EPA's National Study of Chemical Residues in Fish (USEPA, 1992a)
- EPA's Green Bay Mass Balance Study (USEPA, 1992b, 1995c),
- U.S. Department of Agriculture's (USDA) Nutrient Data Bank (Exler, 1987)
- A review from National Marine Fisheries Service of the National Oceanic and Atmospheric Administration (NOAA) (Sidwell, 1981)
- Two California databases (California Toxic Substances Monitoring Program and Bay Protection and Toxic Cleanup Program)

When insufficient data were available from the above sources for certain species, targeted literature searches were conducted and data from primary literature were used. The resulting National lipid database is tabulated in Appendix 5D. The lipid data in this tabulation were carefully screened and reviewed to correct or remove erroneous entries, extreme values of lipid content, and duplicate records.

5.3.4 How Can Site-Specific Organic Carbon Concentrations be Determined?

The concentrations of dissolved and particulate organic carbon (DOC and POC) are used to calculate the freely dissolved fraction of nonionic organic chemicals in water. The concentration of chemical that is freely dissolved is the best measure of the bioavailable concentration of nonionic organic chemicals available for uptake by aquatic organisms. As discussed in Section 3.5.1, sorption of the chemical to DOC and POC reduces chemical bioavailability to aquatic organisms. Bioavailability and the freely dissolved fraction are reduced in waters containing higher concentrations of organic carbon. Therefore, DOC and POC concentrations are important factors for characterizing potential human exposure to nonionic organic chemicals. Baseline BAFs are adjusted for the freely dissolved fraction of chemical in water according to the national BAF methodology, and recalculating a site-specific BAF

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(Method 5, equation 5-17) involves multiplying the baseline BAF by the freely dissolved fraction. This section discusses how the investigator can determine appropriate concentration of DOC and POC in the water column from a site. This can then be used to calculate the freely dissolved fraction, which is used in turn to recalculate a site-specific BAF from a baseline BAF (Method 5).

EPA recommends using local or regional data on the concentrations of DOC and POC in the site water column when recalculating baseline BAFs. The use of such locally or regionally derived (i.e., site-specific) data is encouraged over nationally representative concentrations because local or regional conditions that affect DOC and POC concentrations can differ substantially from those represented by nationally derived values. There is substantial variability in the median values of DOC and POC concentrations in U.S. surface waters USEPA, 2003a). This variability is believed to result from naturally occurring conditions and processes that contribute to spatial and temporal variability in the delivery and biogeochemical cycling of organic carbon in surface waters. Some of these factors include climatology (e.g., arid, arctic, alpine, and tropical zonal differences) and trophic status (e.g., oligotrophic, mesotrophic, and distrophic lakes), discharge volume and source (for streams and rivers), watershed size and landscape characteristics, season, and the extent of tidal influence (for estuaries). In addition, differences among analytical methods used to extract and measure lipids and associated analytical error can contribute to variability in reported values of lipid fraction. To address uncertainty in site-specific BAFs resulting from this natural variability in DOC and POC concentrations, EPA encourages States and authorized Tribes to use appropriate local or regional data on the organic carbon content of applicable waters when adopting criteria into their own water quality standards.

The following sections review the process for calculating the fraction of chemical that is freely dissolved, and offer guidance to the investigator regarding options for determining appropriate organic carbon (DOC and POC) concentrations for site-specific BAF recalculation.

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5.3.4.1 Overview of freely dissolved normalization process

The freely dissolved fraction of nonionic organic chemicals is calculated using equation 3-12 of the 2000 Human Health Methodology (USEPA, 2000a), which requires DOC and POC concentrations appropriate for the site and the octanol-water partition coefficient (K_{ow}) for the chemical of concern:

$$f_{fd} = 1 / (1 + POC \cdot K_{ow} + 0.08 \cdot DOC \cdot K_{ow}) \qquad \text{(Equation 3-6)}$$

Figure 5-12 illustrates the effect of varying concentrations of DOC and POC on the freely dissolved fraction calculated using equation 3-6 as a function of K_{ow}. As this figure illustrates, the calculated freely dissolved fraction is sensitive to organic carbon concentrations for a wide range of chemical hydrophobicities (i.e., $\log K_{ow}$ from 4 to 8.5). For chemicals with $\log K_{ow} > 6.6$, the freely dissolved fraction varies by more than an order of magnitude for organic carbon levels within the 10th to 90th percentile range. The site-specific BAF recalculated by Method 5 (equation 5-15) will change in proportion to the variation in the freely dissolved fraction. Therefore, in order to recalculate an accurate site-specific BAF, it is important for the investigator to determine organic carbon concentrations that are representative of the site.

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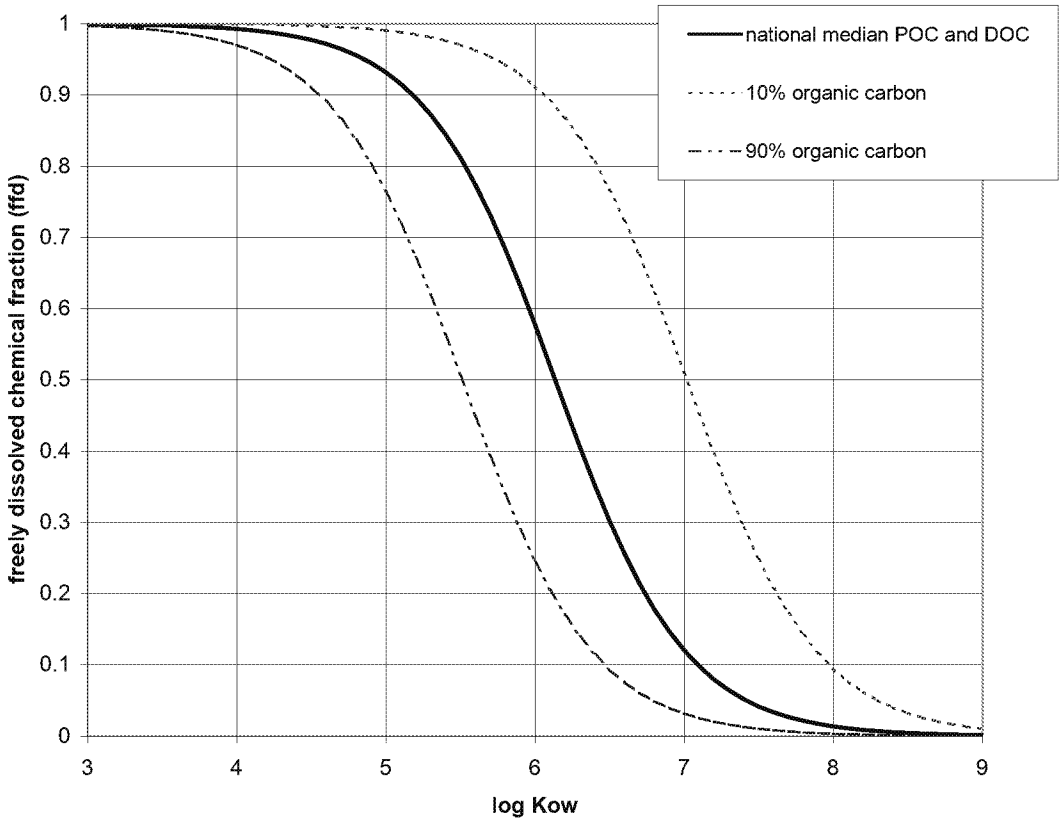

FIGURE 5-12. Illustration of how the freely dissolved fraction calculated using equation 3-12 varies as a function of K_{ow}, for varying concentrations of DOC and POC. Organic carbon concentrations were based on nationally representative data for all waterbody types summarized in Table 5-16. National median values were 2.9 mg/L DOC and 0.5 mg/L POC; 10% values were 1.2 mg/L DOC and 0 POC; 90% values were 9.7 mg/L DOC and 2.3 mg/L POC.

5.3.4.2 Measuring DOC and POC

The investigator may choose to measure organic carbon concentrations in the water column at the site. This would be the preferred approach if site-specific DOC and POC data were not otherwise available. In general, guidance offered in Section 3.5 (*Measuring Chemical Concentrations in Water*) is applicable for designing a field study (e.g., sample numbers, frequency of collection, location, etc.) to measure organic carbon concentrations. Concentrations of DOC and POC in a body of water are expected to vary over time as a function of precipitation events, season, hydrodynamics, and numerous other attributes of a watershed. Thus, sufficient sampling of DOC and POC concentrations over space and time is needed to achieve

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representative estimates for calculating freely dissolved chemical fractions . The sampling and averaging of DOC and POC concentrations should follow the guidance for field studies to measure chemical concentrations in water (Section 3.5) as well as the guidance in Section 3.3.3.4 regarding how optimization of water sampling depends on the target chemical's hydrophobicity. This is especially important for highly hydrophobic chemicals because the impact of DOC and POC on the site-specific BAF recalculation is greatest with these chemicals. The guidance offered in this section specifically relates to the separation of organic carbon into DOC and POC fractions and the selection of analytical methods for measuring organic carbon.

The separation of POC from DOC in water samples is operationally defined by filtering or centrifugation. With both techniques, the distinction between POC and DOC is usually defined in terms of a particle size cutoff, which can differ depending upon membrane selection and hardware. For example, a membrane with a 0.45- m cutoff may be used in one study, whereas centrifugation that retains all particles with a size of 1.0 m or greater may be used in another study. Typically, the particle size cutoff between POC and DOC fractions is 0.1-1 m. DOC is principally composed of carbohydrates, carboxylic acids, amino acids, hydrocarbons, hydrophilic acids, and humic and fulvic acids. POC is principally composed of some larger humic acids, microbes, small plankton, plant litter, and ligneous matter (Suffet et al. 1994; Thurman, 1985). The material retained by filtration or centrifugation is the POC fraction. The DOC fraction is defined as the ambient water remaining after filtration or centrifugation is performed. Total organic carbon (TOC) is the sum of the POC and DOC fractions.

Two methods are commonly used to measure dissolved and particulate organic carbon concentrations. The first (and preferred) method is to perform organic carbon analyses on the DOC and POC fractions of the same samples. This method should be chosen unless the concentration of organic carbon is too low to quantify in one of the fractions (typically this would be the POC fraction, because POC concentrations are usually lower than DOC in surface waters). If organic carbon cannot be quantified in the POC fraction, the investigator can have the organic carbon analysis performed on the TOC (i.e., whole water) and DOC fractions of the samples. In this method, POC concentrations are determined by the difference between TOC and

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DOC (i.e., POC = TOC - DOC). The disadvantage of calculating POC by difference is that the result will be less precise than the analytical methods.

Several different methods are available for analyzing organic carbon in water samples. The persulfate-ultraviolet oxidation method (*Standard Methods* #5310C) is generally preferred to the wet oxidation method (*Standard Methods* #5310D), because the former method is more sensitive and has a significantly lower detection limit. EPA's approved method for organic carbon analysis in water samples (EPA SW-846 Method 9060A) allows for either of these methods to be used. The errors associated with different analytical methods for organic carbon appear to be small, relative to other sources of variability and uncertainty in DOC and POC data (USEPA, 2003b).

5.3.4.3 Determining DOC and POC using a literature or database search

Scientific publications, reports, and online databases also contain data for organic carbon concentrations measured in many ecosystems. As was the case for lipid content, determining DOC and POC concentrations in this manner may be an economical and expedient alternative to field measurement, if the appropriate data can be found. Large electronic databases of aquatic chemistry and ecosystem data that can be accessed via the Internet are probably the best available resource for organic carbon data. Not only are they used as repositories and clearinghouses for aquatic environmental data for many aquatic organisms and ecosystems, but search and retrieval of specific data from these databases is generally straightforward.

The following is an inventory and descriptions of Internet-accessible databases containing organic carbon data for many ecosystems, waterbodies and locations in the United States. The databases include:

- Environmental Monitoring and Assessment Program (EMAP)
- STORET (STOrage and RETrieval) and Legacy Data Center (LDC) and
- National Water Information System (NWIS)

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Descriptions of each database, and how DOC and POC data can be accessed from each, are provided in Appendix 5B.

5.3.4.4 How should organic carbon data be evaluated?

Organic carbon data acquired from the databases described above, or other sources, should be evaluated by the investigator in terms of their usability for establishing DOC and POC values to be used for calculating freely dissolved chemical fractions and subsequently recomputing site-specific BAFs. Table 5-11 provides a list of evaluation criteria for organic carbon data sources. Because a "site" may be variously defined as a particular waterbody or segment, a type of waterbody within a state or region, or even all waterbodies within a state or region, this guidance should be flexible in order to properly address each situation. The order of the criteria generally corresponds to their importance in the evaluation process. The three top entries in Table 5-11 *(waterbody types of interest, QA/QC information,* and *sample factors influencing variability*) are considered to be essential information. Organic carbon data must be available for the site waterbody type(s) in order to be usable. QA/QC and sampling data should also be available for the investigator to evaluate the validity and representativeness of the data.

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Table 5-11. Evaluation Criteria for Organic Carbon Data Sources

Evaluation Criteria for Organic Carbon Data Sources:
Waterbody type(s) of interest
QA/QC information: • analytical methods (including whether POC was determined by difference; i.e., TOC - DOC) • detection limits • % recovery • relative standard deviation (RSD)
Sample factors influencing variability: • watershed ecoregion, size and land use • waterbody type and trophic status • spatial/temporal representativeness and bias • station type ("ambient" only) • hydrograph • tidal influence
Data quantity
Sampling period (collected since 1980)
Occurrence of extreme values

For example, station types should be restricted to "ambient" sampling stations only, to exclude so-called specialty stations (i.e., those stations designated for special purposes such as storm water runoff and biological and sediment monitoring). When POC and/or DOC concentrations are reported to be below analytical detection levels, it may be appropriate to estimate the concentration value as half of the reported detection level. However, the investigator should consider discarding censored data with "high" detection levels (i.e., >1.0 mg/L for DOC and >0.2 mg/L for POC) because of the greater uncertainty involved in estimating definitive values of DOC and POC in these situations. Finally, in cases where the parameter of interest (POC or DOC) must be calculated as the difference from two other measurements (i.e., POC = TOC – DOC; DOC = TOC – POC), the calculation should only be performed using data from the same sample to avoid introducing error, and the results should be screened to remove negative organic carbon concentrations.

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Data quantity and *occurrence of extreme values* are useful criteria in terms of evaluating the representativeness of organic carbon data. *Sampling period* is also included as a factor in Table 5-11, as it may be appropriate to eliminate data collected prior to implementation of secondary wastewater treatment (e.g. 1980) because of the greater uncertainty in using pre-secondary treatment era data to represent present-day conditions that can affect organic carbon concentrations in surface waters.

5.3.4.5 Determining organic carbon concentrations using the National DOC/POC Database

The investigator may also determine DOC and POC concentrations by selecting values from the database for surface water organic carbon developed by EPA during the development of the National BAF TSD Volume 2 (USEPA; 2003). Information on organic carbon concentrations representative of different types of surface waters was obtained for the national database from a variety of primary and secondary sources. Data on the concentrations of DOC and POC in U.S. surface waters were obtained from two databases:

- The U.S. Geological Survey's (USGS) WATSTORE database
- EPA's historical STORET database (recently renamed the Legacy Data Center [LDC] database)

Numerous steps were then taken to process and screen the DOC and POC data so that only the most appropriate data would be retained for calculating the national default values (USEPA, 2003). The screening steps included: deletion of suspect or uncertain values; restriction to samples collected in the following waterbody types: estuaries, lakes, reservoirs, and streams (including rivers); elimination of pre-1980 data ; and, removal of extreme values based on the criteria of Thurman (1985).

Table 5-12 shows descriptive statistics surrounding the median values for DOC and POC, in addition to values for specific waterbody types. It is evident from Table 5-12 that the variation in DOC and POC concentrations is relatively large. For example, with the exception of estuaries,

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the coefficient of variations around the means are all above 100% and approach or equal 200% in some cases. Ratios of the 95th to the 5th percentiles range from a factor of 5 to 30, depending on waterbody type and parameter. This variation is not unexpected, given the high degree of temporal and spatial heterogeneity represented in the database. It is also apparent that the type of waterbody (lake, stream, estuary) has some impact on the DOC and POC distributions. For example, median values of DOC and POC from samples designated as "stream/river" are nearly twice those designated as "lakes." This difference is probably related to the differing hydrologic, biogeochemical, and watershed characteristics of streams and lakes. Given the relatively high degree of variation that is evident in DOC and POC concentrations in surface waters across the United States, EPA recommends that States and Tribes consider deriving appropriate values of DOC and POC by using local or regional data (as described in the previous two sections) when sufficient data are available. If local or regional values cannot be derived, then it may be appropriate to use conservative (i.e., 90 or 95th percentile) values for DOC and POC concentrations listed in Table 5-12, when calculating the freely dissolved chemical fraction for site-specific BAF recalculation.

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Table 5-12. National Default Values for POC and DOC in U.S. Fresh and Estuarine Surface Waters

Statistic	DOC (mg/L)				POC (mg/L)			
	All Types	Stream/ River	Lake/ Reservoir	Estuary	All Types	Stream/ River	Lake/ Reservoir	Estuary
Median	2.9	3.8	2.1	2.7	0.5	0.6	0.3	0.9
Mean	4.6	5.6	2.9	3.4	1.0	1.3	0.5	1.2
Std.	5.1	5.9	3.0	2.6	2.0	2.5	1.0	1.8
CV	111%	105%	103%	76%	200%	192%	200%	150%
n	111,059	69,589	25,704	15,766	86,540	48,238	23,483	14,819
5th	0.8	0.7	1.0	1.7	0a	0a	0.08	0.1
10th	1.2	1.0	1.4	2.0	0	0a	0.1	0.3
25th	2.0	2.1	1.8	2.3	0.2	0.2	0.2	0.5
75th	5.4	6.9	2.6	3.2	1.1	1.4	0.5	1.4
90th	9.7	11.6	5.0	5.0	2.3	3.1	0.8	2.2
95th	14	16.5	7.8	9	3.9	5	1.3	3
95th/5th	17.5	23.6	7.8	5.3	—	—	16.3	30.0

[a] Values calculated to be less than zero because of measurement error
Source: U.S. EPA LDC and USGS WATSTORE databases. Data retrieval: January 2000

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ED_006490_00009970-00356

Appendix 5A

Internet-Accessible Databases Containing Lipid Content Data

EMAP

EMAP is an EPA research program to develop tools to monitor and assess the status and trends of national ecological resources. EMAP aims to advance the science of ecological monitoring and ecological risk assessment, guide national monitoring with improved scientific understanding of ecosystem integrity and dynamics, and demonstrate multi-agency monitoring through large regional projects. EMAP data are organized according to these projects, which include 5 major surface water projects and a number of smaller regional EMAP (REMAP) projects. EMAP data are accessible via the EPA web site, at http://www.epa.gov/emap.

ACCESSING EMAP LIPID CONTENT DATA

1. Access the EMAP web site on the internet at http://www.epa.gov/emap/
2. Click on DATA
3. Click on the EMAP data directory (http://osapub.epa.gov/emap/emap.search)

Although the Data Set Search Engine accessed at this stage lists "% LIPIDS" as a keyword, making this selection returns lipid data from only one project, the *1993-1994 REMAP Region 1 Fish Tissue Organic Concentrations by Composite data set*. A better approach is to first select a regional project, select **view data** for the appropriate dataset of that project [e.g., "Tissue Data (Organics)"], and then browse to see if there are any lipid data available. This can be done by searching for the "LIPID" parameter in the "Variables" list that appears at the top of each dataset output. This output is formatted as comma-separated value (CSV) text, which can be saved from a web browser and then opened in a spreadsheet. At the time of this report, there seems to be no other good, direct way to search EMAP for lipid content data with any reliability or certainty of completeness. This is unfortunate, because EMAP data sets have the most complete supporting information of any of the online databases. Many of the EMAP data sets

ED_006490_00009970-00357

have an accompanying documentation and references that provide information about the study design, sampling procedures, analytical methods, and QA procedures.

NWIS

NWIS is a database system (48 separate NWIS databases nationwide) maintained by the US Geological Survey to provide access to current and historical water-resources data collected by the Survey at approximately 1.5 million sites in all 50 States, the District of Columbia, and Puerto Rico. The NWISWeb web site can be used to search for and retrieve NWIS data by category (e.g., surface water, ground water, or water quality) and by geographic area. The NWISWeb is accessed at http://waterdata.usgs.gov/nwis/qw.

ACCESSING NWISWEB LLIPID CONTENT DATA

1. Access NWISWeb on the internet at http://waterdata.usgs.gov/nwis/qw
2. Click on "Samples"
3. Select the type of geographic area you wish to search (state, hydrologic region (HUC) or latitude-longitude box)
4. Select "Sample medium type" and "Parameter groupings" under "Data Attribute"
5. Select "Geographic region" desired in first list
6. Select [C] "Animal tissue" in second list
7. Select "Organics" in third list
8. Under "Output Format," choose a Tab-separated data file
9. Press "Submit"
10. Save output as a text file and open as a tab-separated text file in spreadsheet
11. Sort the data by lipid code (49289: % lipids in whole organism, or 63595: % lipids in tissue)

NWISWeb contains a significant amount of lipid content data collected by the USGS. The data can be searched by geographic area and by parameter group (lipids are found in the organics parameter group), but not by a specific parameter. Once the search is performed as described in the text box above, all the available lipid content data for the particular geographic area will be obtained.

There is limited supporting data available online in the NWISWeb database. Field and laboratory protocols, changes in those protocols, and other QA/QC information are documented

ED_006490_00009970-00358

in numerous reports and technical memoranda. These range from project-specific reports to national protocols. Specific analytic procedures or codes are not identified for the lipid content data in the NWISWeb database. The lack of supporting information, combined with the difficulty of searching for lipid content as a specific parameter (versus manually searching within the data for the entire organics parameter group) limits the utility of this database.

STORET and LDC

EPA maintains two data management systems containing water quality information for the nation's waters: the Legacy Data Center (LDC), and STORET. The LDC is a static, archived database and STORET is an operational system actively being populated with water quality data. The LDC contains historical water quality data dating back to the early part of the 20th century and collected up to the end of 1998. STORET contains data collected beginning in 1999, along with older data that has been properly documented and migrated from the LDC. Both systems contain raw biological, chemical, and physical data on surface and ground water collected by federal, state and local agencies, Indian Tribes, volunteer groups, academics, and others. All 50 States, territories, and jurisdictions of the U.S. are represented in these systems.

Each sampling result in the LDC and in STORET is accompanied by information about where the sample was taken (latitude, longitude, state, county, Hydrologic Unit Code and a brief site identification), when the sample was collected, the medium sampled (e.g., water, sediment, fish tissue), the monitoring organization, and the sampling and analytical methods used. Both the LDC and STORET are accessible via the Internet at http://www.epa.gov/storet/dbtop.html. The following are detailed instructions for accessing lipid data from the LDC:

ACCESSING THE LDC FOR LIPID CONTENT DATA

1. Select "Browse" or "Download Legacy STORET Data"
2. Select the link for the "Advanced Query Form"
3. Under "Station type," check the "Surface water" box
4. Select either the organization or the desired geographic location by Latitude/Longitude, State/County (only one county can be selected at a time), or HUC.
5. Enter sampling data range, if desired (optional)
6. Enter the desired parameter codes (these can be looked up using the "Search by name"

ED_006490_00009970-00359

box): 49289 for lipids
7. Click the "Done" button under "Submit Form"
8. Select a detailed report in HTML format and press "Continue"

The investigator can also make advanced searches of geographic locations as well as specific parameters in the LDC. If data from more than 50 stations are retrieved from within the search results, the information is emailed to the investigator and cannot be viewed immediately. In this case, a link that can be accessed to download the data is emailed in approximately 24 hours (extremely large files may take slightly longer).

To access lipid data from STORET, the investigator can follow these instructions:

ACCESSING STORET FOR LIPID CONTENT DATA

1. Select "Browse" or "Download Modernized STORET Data"
2. Under "STORET Biological Results," select the link for "Biological results by geographic location"
3. Select the desired geographic location by State and County, Latitude/Longitude box, HUC, etc; (the entire data set can also be selected)
4. Select a sampling date range, if desired (optional)
5. Select "Fish/Nekton" under the "Community Sampled" box
6. In the "Characteristic" search box, enter "Lipid" and click the search button
7. In the pop-up window which appears, click on "Lipid (unspecified mix)" and then click the "Select" button
8. This selection will be placed into the "Characteristic name" box on the search page; press "Continue"
9. The "Results Search Summary" shows the number of data that were found
10. To download the results, press "Continue" and after the search engine has completed the search (which may take as number of minutes for large data sets), click the appropriate link to download the data
11. After the download is completed, the data will be displayed in a text format
12. Save the file as a "Text File"
13. Open the file in a spreadsheet program (such as Microsoft Excel) as a "Text File;" you will need to indicate that this is a file delimited using the "~" character (in Excel, click on the box for "Other" under delimiters and type the ~ symbol,, which is found on the keyboard over the accent symbol, next to number 1, then click "Finish")
14. If the retrieval succeeded, a new spreadsheet should be opened with your data. Some headings may be slightly off and some "chunks" of data may be displaced, so review and editing of the data may be necessary.

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There are a number of differences between the LDC and STORET that should be considered by the investigator searching for lipid content data. LDC contains a huge quantity of data reported prior to 1999. However, the quality of some of this information is suspect and the supporting information may be incomplete or missing. STORET is a superior database in terms of data access, quality, and completeness of supporting information. STORET provides documentation of data quality, in the form of reports, which describe the standards, methods, practices, and other metadata supplied by data owners to document the quality of the monitoring results found in STORET. At this time, it holds significantly less data (as of January 2006, the lipid retrieval from STORET documented above returned 959 results), reported since 1999.

U.S. Army Corps of Engineers BSAF and Lipid Database

The *Biota-Sediment Accumulation Factor Database* is an internet-accessible database (http://el.erdc.usace.army.mil/bsaf/BSAF.html) maintained by the Engineer Research and Development Center (ERDC) of the U.S. Army Corps of Engineers. This database contains both BSAF and organism lipid content data, primarily obtained from peer reviewed journal articles. All data are documented to the original reference, and include information about the tissue sampled, the number of measurements, and any available error statistics. The BSAF database contains lipid data for over 300 aquatic species and other groups, which can be selected via pull-down menus and/or viewed in tables. The BSAF database web site also provides simple instructions for downloading and transferring data in to a spreadsheet program.

EPA's Office of Water National Lake Fish Tissue Study

The *National Study of Chemical Residues in Lake Fish Tissue* was a screening-level study designed to estimate the national distribution of selected persistent, bioaccumulative, and toxic (PBT) chemicals in fish tissue from lakes and reservoirs of the continental United States. The study involves the collection of predator and bottom-dwelling fish from 500 randomly selected lakes and reservoirs of the continental United States (excluding the Great Lakes) over a period of four years (~125 lakes per year), commencing in 1999 and 2000. The selection of target fish

ED_006490_00009970-00361

species followed EPA guidance (USEPA, 1995b). Samples were edible tissue (skin-on fillet) composites of targeted predator species and total body tissue (whole fish) composites of targeted bottom-dwelling species.

Lipid contents were determined for all samples using the procedure described in EPA Methods 1613B and 1668A, the same procedure used in EPA's National Dioxin Study. Consistent field and laboratory QA procedures were followed throughout the study, and are well documented. Although EPA maintains a Web site for this project (http://www.epa.gov/waterscience/fishstudy/), the data are currently being released on CDs that contain the results of quality-assured raw data in large spreadsheet files. The CDs can be ordered by contacting the National Lake Fish Tissue Study Manager, whose contact information is provided on the study web site.

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Appendix 5B

Internet-Accessible Databases Containing Organic Carbon Data

EMAP

EMAP data are accessible via the EPA web site, at http://www.epa.gov/emap. As discussed in Appendix 5A, the preferred approach to access this database is to first select a regional project, select **view data** for the appropriate dataset of that project (i.e., Water Chemistry Data"), and then browse to see if there are any organic carbon data available. This can be done by searching for the "ORGANIC CARBON" parameter in the "Variables" list that appears at the top of each dataset output. Following this approach, DOC data were found in the following regional projects and datasets:

- 1991-1994 Northeast Lakes, Water Chemistry Data Summarized by Lake
- 1993-1994 Region 1, Lake Dissolved Organic Carbon Data Set
- 1993-1996 Mid-Atlantic Streams, Water Chemistry
- 1994-1995 Region 10, Validated Water Chemistry Data
- 1997-1998 MAIA Streams, Validated Water Chemistry

No data were found for POC or TOC concentrations in the EMAP project datasets.

STORET and LDC

Both the LDC and STORET are accessible via the Internet at http://www.epa.gov/storet/dbtop.html. To access DOC and POC data from the LDC, the investigator should follow the following instructions:

ED_006490_00009970-00363

ACCESSING THE LDC FOR ORGANIC CARBON CONTENT DATA

1. Select "Browse" or "Download Legacy STORET Data"
2. Select the link for the "Advanced Query Form"
3. Under "Station type," check the "Surface Water" box
4. Select either the organization or the desired geographic location by Latitude/Longitude, State/County (only one county can be selected at as time), or HUC
5. Enter sampling date range, if desired (optional)
6. Enter the desired parameter codes (these can be looked up using the "Search by name" box): 00680 and 00690 for TOC, 00681 and 00684 for DOC, 00689 and 80102 for POC
7. Click the "Done" button under "Submit Form"
8. Select a detailed data report in HTML format and press "Continue"

The LDC contains a large quantity of data reported prior to 1999, however the quality of some of this information is suspect, and the supporting information may be incomplete or missing. For the LDC data, the analytical methods used to determine DOC and POC concentrations were not reported in the database. As EPA notes on the STORET web site, all data owned by Agency "112WRD" (USGS) have been removed from the LDC. This resolves a problem of duplicate data appearing in both EPA (LDC) and USGS (NWIS) databases that previously confronted users of both systems.

To access organic carbon data from STORET, the investigator can follow these instructions:

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ACCESSING STORET FOR ORGANIC CARBON DATA

1. Select "Browse" or "Download Modernized STORET Data"
2. Under "STORET Regular Results," select the link for "Regular results by geographic location"
3. Select the desired geographic location by State and County, Latitude/Longitude box, HUC, etc; (the entire data set can also be selected)
4. Select a sampling date range, if desired (optional)
5. Select "Water" under the "Activity Medium" box
6. In the "Characteristic" search box, enter "Carbon" and click the "Search" button
7. In the pop-up window which appears, click on "Carbon, organic" and then click the "Select" button
8. This selection will be placed into the "Characteristic" name box on the search page; press "Continue"
9. The "Results Search Summary" shows the number of data that were found
10. To download the results, press "Continue" and after the search engine has completed the search (which may take a number of minutes for large data sets), click the appropriate link to download the data
11. After the download is completed, the data will be displayed in a "Text" format
12. Save the file as a "Text" file
13. Open the file in a spreadsheet program (such as Microsoft Excel) as a "Text" file; you will need to indicate that this is a file delimited using the "~" character (in Excel, click on the box for "Other" under delimiters and type the "~" symbol, which is found on the keyboard over the accent symbol, next to number 1, then click "Finish")
14. If the retrieval succeeded, a new spreadsheet should be opened with your data. Some headings may be slightly off and some "chunks" of data may be displaced, so review and editing of the data may be necessary.

STORET also holds a significant amount of organic carbon data. As of January 2006, the organic carbon retrieval from STORET documented above returned 44,962 results for data reported since 1999. As previously noted, STORET provides documentation of data quality, in the form of reports which document the standards, methods, practices, and other metadata supplied by data owners to document the quality of the monitoring results found in STORET. A PDF file that details the analytical remark codes found in the "Analytical Proc. ID" output can be found at http://www.epa.gov/storet/modern/doc/FieldLabAnltPrcdAndEqpDetail.pdf .

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NWIS

NWIS also contains a large amount of organic carbon data for surface waters in the United States. The NWISWeb is accessed at http://waterdata.usgs.gov/nwis/qw. Details regarding access of NWIS to obtain organic carbon data are provided below:

ACCESSING NWIS WEB ORGANIC CARBON DATA

1. Access NWISWeb on the internet at: http://waterdata.usgs.gov/nwis/qw
2. Click on "Samples"
3. Select the type of geographic area you wish to search (state, hydrologic region (HUC), or latitude-longitude box)
4. Select "Sample Medium Type" and "Parameter Groupings" under "Data Attribute"
5. Select "Geographic Region" desired in first list
6. Select [9] "Surface water" in second list
7. Select "Major Inorganics" in third list
8. Under "Output Format," choose a tab-separated data file
9. Press "Submit"
10. Save output as a "Text" file and open as a tab-separated text file in spreadsheet
11. Sort the data by "Organic Carbon" codes:
    - 00689 – Organic carbon, suspended sediment, total, milligrams per liter
    - 00681 – Organic carbon, water, filtered, milligrams per liter
    - 00680 – Organic carbon, water, unfiltered, milligrams per liter

For NWIS data, estimates of accuracy (percent recovery) and precision (relative standard deviation) are available for the analysis of TOC and POC. In general, however, the lack of supporting information, combined with the difficulty of searching for organic carbon as a specific parameter (versus manually searching within the data for the entire "major inorganics" parameter group) limits the utility of this database.

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Appendix 5C

BSAFs for PCB congeners in Green Bay and Upper Hudson River

Table 5C-1. BSAFs for PCB congeners based on measurements made in Green Bay, Lake Michigan. Average, coefficient of variation (CV), minimum and maximum BSAFs for each congener across all zones, age classes and fish species are tabulated.

PCB CONGENER	LOG K_{OW}	AVERAGE BSAF	BSAF CV	MINIMUM BSAF	MAXIMUM BSAF
PCB 5+8	5.02	0.264	1.16	0.0536	1.17
PCB 6	5.06	0.405	0.489	0.140	0.728
PCB 7	5.07	0.362	0.294	0.231	0.500
PCB 16+32	5.30	0.767	0.587	0.189	1.98
PCB 17	5.25	3.11	1.55	0.274	16.0
PCB 18	5.24	1.91	1.07	0.171	8.41
PCB 19	5.02	0.66	0.347	0.303	1.06
PCB 22	5.58	1.04	1.23	0.148	4.66
PCB 24+27	5.40	2.34	1.13	0.362	9.79
PCB 25	5.67	1.54	0.949	0.179	5.59
PCB 26	5.66	1.78	1.01	0.204	7.40
PCB 28+31	5.67	1.56	0.705	0.229	4.15
PCB 29	5.60	0.224	0.000	0.224	0.224
PCB 33	5.60	0.686	0.937	0.108	2.39
PCB 37+42	5.80	0.876	0.487	0.318	1.44
PCB 40	5.66	1.66	0.598	0.618	4.05
PCB 41+64+71	5.87	1.90	0.585	0.541	4.32
PCB 43	5.75	7.25	0.508	2.84	15.9
PCB 45	5.53	2.84	0.655	0.597	7.47
PCB 46	5.53	1.03	0.614	0.398	3.05
PCB 47+48	5.82	10.3	1.08	0.544	36.0
PCB 49	5.85	5.60	1.17	0.777	27.9
PCB 52	5.84	7.65	1.12	1.02	34.53
PCB 53	5.62	2.39	0.404	0.976	4.18
PCB 56+60	6.11	2.00	0.867	0.288	8.62
PCB 63	6.17	4.12	0.614	1.09	9.10
PCB 66+95	6.17	4.72	0.719	0.469	16.3
PCB 70+76	6.17	2.05	1.04	0.257	10.7
PCB 74	6.20	3.63	0.816	0.614	14.1
PCB 77+110	6.42	5.05	0.889	0.740	21.6
PCB 81	6.36	11.4	0.474	3.68	28.3
PCB 82	6.20	6.09	0.987	0.806	25.5
PCB 83	6.26	8.98	0.647	2.47	28.3
PCB 84+92	6.20	4.77	0.898	1.02	16.5
PCB 85	6.30	7.38	0.710	1.03	25.8
PCB 87	6.29	7.62	0.885	0.907	30.0
PCB 89	6.07	4.79	0.560	1.97	9.09
PCB 91	6.13	6.78	0.747	1.16	19.9
PCB 97	6.29	7.20	0.760	0.966	24.2
PCB 99	6.39	6.12	0.623	1.45	18.1
PCB 100	6.23	1.58	0.487	0.563	2.61

ED_006490_00009970-00367

Table 5C-1 (continued). BSAFs for PCB congeners based on measurements made in Green Bay, Lake Michigan. Average, coefficient of variation (CV), minimum and maximum BSAFs for each congener across all zones, age classes and fish species are tabulated.

PCB CONGENER	LOG K_{OW}	AVERAGE BSAF	BSAF CV	MINIMUM BSAF	MAXIMUM BSAF
PCB 101	6.38	8.39	0.623	1.18	23.0
PCB 105+132+153	6.72	6.06	0.828	0.913	25.3
PCB 107	6.71	7.12	0.783	1.62	25.6
PCB 114+134	6.60	156	1.10	7.94	497
PCB 118	6.74	5.74	0.808	0.772	20.0
PCB 119	6.58	2.89	0.546	0.398	7.17
PCB 124+135+144+147	6.67	6.04	0.833	1.14	17.8
PCB 128	6.74	9.29	0.546	2.92	22.2
PCB 129+178	6.94	5.56	0.742	0.970	18.9
PCB 130	6.80	11.2	0.750	1.62	31.3
PCB 131	6.58	1.63	0.366	0.970	2.53
PCB 136	6.22	6.65	1.63	0.199	19.1
PCB 138+158+163	6.95	11.07	0.765	1.19	40.84
PCB 141 (+179)	6.78	12.19	0.860	1.13	42.94
PCB 146	6.89	8.76	1.13	0.236	32.59
PCB 149	6.67	8.68	0.971	0.889	38.15
PCB 151	6.64	7.11	0.596	2.25	18.13
PCB 156+171+202	7.18	26.34	0.501	9.94	48.12
PCB 167	7.27	13.41	0.697	3.06	40.58
PCB 170+190	7.37	5.78	1.03	0.95	22.41
PCB 172+197	7.32	23.03	0.671	4.861	64.51
PCB 174	7.11	6.30	0.867	1.09	25.9
PCB 175	7.17	6.06	0.652	1.91	18.7
PCB 177	7.08	9.05	0.864	1.50	26.7
PCB 180	7.36	11.85	1.05	0.722	50.0
PCB 182+187	7.19	7.36	0.931	0.635	27.5
PCB 183	7.20	7.22	0.812	1.56	25.1
PCB 189	7.71	8.45	0.280	5.66	14.2
PCB 191	7.55	34.93	0.953	5.23	127
PCB 193	7.52	13.98	0.928	1.83	49.2
PCB 194	7.80	2.60	0.620	0.428	6.97
PCB 195+208	7.64	1.01	0.991	0.0626	3.86
PCB 196+203	7.65	8.70	0.981	1.65	33.2
PCB 198	7.62	0.35	0.676	0.196	0.876
PCB 201	7.62	6.05	0.651	2.21	14.8
PCB 200	7.27	6.50	0.937	1.15	25.0
PCB 206	8.09	1.44	1.08	0.178	5.98
PCB 207	7.74	0.68	0.672	0.117	1.70
PCB 209	8.18	0.31	1.10	0.0788	1.25
AVERAGE (ALL CONGENERS)		**7.85**	**0.790**	**1.28**	**25.4**

ED_006490_00009970-00368

Table 5C-2. BSAFs for PCB congeners based on measurements made in the Hudson River. Average, coefficient of variation (CV), minimum and maximum BSAFs for each congener across all zones, age classes and fish species are tabulated.

PCB CONGENER	LOG K_{OW}	AVERAGE BSAF	BSAF CV	MINIMUM BSAF	MAXIMUM BSAF
PCB 1	4.46	0.149	0.774	0.0594	0.406
PCB 3	4.69	0.0340	0	0.0340	0.0340
PCB 4	4.65	0.407	0.933	0.0324	1.19
PCB 6	5.06	0.144	0.977	0.00668	0.360
PCB 8	5.07	0.250	1.02	0.00851	0.733
PCB 9	5.06	0.121	1.02	0.0173	0.296
PCB 10	4.84	0.655	1.19	0.0471	3.15
PCB 15	5.30	0.0882	0.956	0.00314	0.220
PCB 16	5.16	0.570	0.734	0.0769	1.30
PCB 17	5.25	1.85	0.274	1.47	2.57
PCB 18	5.24	1.19	0.876	0.123	3.75
PCB 19	5.02	0.877	0.810	0.0480	2.14
PCB 20	5.57	3.61	0.177	3.09	4.32
PCB 22	5.58	1.53	0.552	0.496	3.12
PCB 25	5.67	1.08	0.733	0.0952	2.73
PCB 26	5.66	1.62	0.713	0.124	4.10
PCB 27	5.44	1.58	0.420	0.924	2.16
PCB 28	5.67	2.34	0.586	0.918	5.81
PCB 31	5.67	1.71	0.729	0.171	4.34
PCB 32	5.44	1.43	0.963	0.0409	5.38
PCB 33	5.60	2.46	0.254	1.91	3.15
PCB 34	5.66	1.17	1.03	0.0706	4.21
PCB 37	5.83	0.469	0.867	0.0870	1.31
PCB 40	5.66	1.82	0.623	0.172	3.60
PCB 41	5.69	1.40	1.01	0.247	2.98
PCB 42	5.76	5.01	0.198	4.12	6.19
PCB 44	5.75	4.63	0.521	1.75	11.5
PCB 45	5.53	3.07	0.139	2.59	3.50
PCB 47	5.85	3.99	0.778	0.157	12.1
PCB 48	5.78	4.05	0.448	1.86	6.65
PCB 49	5.85	5.30	0.747	0.361	16.0
PCB 51	5.63	2.95	0.942	0.0635	10.7
PCB 52	5.84	5.24	0.725	0.468	15.2
PCB 53	5.62	1.82	0.492	0.370	2.88
PCB 56	6.11	4.09	0.574	1.24	9.93
PCB 60	6.11	4.99	0.689	1.30	13.2
PCB 63	6.17	6.01	0.356	3.08	8.95
PCB 64	5.95	5.83	0.884	0.835	21.5
PCB 66	6.20	6.67	0.508	2.67	14.9
PCB 67	6.20	63.6	0.584	12.4	119
PCB 70	6.20	6.54	0.564	2.53	15.4
PCB 74	6.20	7.78	0.167	6.33	9.48
PCB 77	6.36	1.92	0.767	0.295	4.71
PCB 82	6.20	7.58	0.731	3.67	15.6

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Table 5C-2 (continued). BSAFs for PCB congeners based on measurements made in the Hudson River. Average, coefficient of variation (CV), minimum and maximum BSAFs for each congener across all zones, age classes and fish species are tabulated.

PCB CONGENER	LOG K_{OW}	AVERAGE BSAF	BSAF CV	MINIMUM BSAF	MAXIMUM BSAF
PCB 83	6.26	6.53	0.444	3.38	12.6
PCB 84	6.04	3.98	0.835	0.180	12.8
PCB 85	6.30	11.1	0.525	5.24	26.3
PCB 87	6.29	7.82	0.332	4.03	12.3
PCB 101 + 90	6.37	9.01	0.496	2.65	21.3
PCB 91	6.13	6.03	0.725	0.285	13.4
PCB 92	6.35	8.40	0.758	0.981	24.2
PCB 95	6.13	5.23	0.805	0.271	15.0
PCB 96	5.71	3.62	0.104	3.19	3.90
PCB 97	6.29	8.81	0.418	4.98	16.7
PCB 99	6.39	11.1	0.526	3.11	27.5
PCB 105	6.65	10.5	0.322	7.83	15.3
PCB 107	6.71	13.6	0.414	7.34	25.2
PCB 110	6.48	9.04	0.200	7.38	11.5
PCB 114	6.65	20.9	0.459	6.68	38.5
PCB 118	6.74	10.9	0.454	4.14	18.8
PCB 119	6.58	14.7	0.397	9.37	22.3
PCB 122	6.64	15.5	0.447	10.6	20.5
PCB 123	6.74	5.90	0.105	5.46	6.34
PCB 128	6.74	11.0	0.552	4.87	23.0
PCB 129	6.73	7.41	0.123	6.37	8.10
PCB 135	6.64	19.1	0.266	12.8	25.0
PCB 136	6.22	8.48	0.485	3.51	15.0
PCB 137	6.83	13.5	0.523	8.25	21.5
PCB 138	6.83	9.96	0.575	2.13	23.3
PCB 141	6.82	10.8	0.516	5.83	24.9
PCB 144	6.67	24.5	0.681	4.46	59.4
PCB 146	6.89	15.1	0.635	6.63	38.5
PCB 149	6.67	8.69	0.717	0.689	22.4
PCB 151	6.64	8.55	0.136	7.73	9.37
PCB 153	6.92	13.6	0.529	7.33	30.3
PCB 156	7.18	13.6	0.402	8.52	21.3
PCB 158	7.02	13.1	0.492	8.21	27.5
PCB 167	7.27	13.6	0.493	8.60	28.6
PCB 170	7.27	12.4	0.644	6.14	30.1
PCB 172	7.33	11.7	0.524	6.84	23.5
PCB 174	7.11	13.0	0.396	7.77	20.0
PCB 177	7.08	10.3	0.565	4.28	22.4
PCB 178	7.14	1.85	0.274	1.47	2.57
PCB 180	7.36	12.5	0.608	6.48	28.4
PCB 183	7.20	13.0	0.0276	12.6	13.2

ED_006490_00009970-00370

Table 5C-2 (continued). BSAFs for PCB congeners based on measurements made in the Hudson River. Average, coefficient of variation (CV), minimum and maximum BSAFs for each congener across all zones, age classes and fish species are tabulated.

PCB CONGENER	LOG K_{OW}	AVERAGE BSAF	BSAF CV	MINIMUM BSAF	MAXIMUM BSAF
PCB 187	7.17	16.9	0.727	7.18	49.4
PCB 201	7.62	14.7	0.531	7.80	30.4
PCB 202	7.24	11.0	0.356	8.20	13.7
PCB 203	7.65	11.5	0.691	5.21	31.4
AVERAGE (ALL CONGENERS)		**7.66**	**0.565**	**3.52**	**15.4**

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Appendix 5D

Lipid Content of Aquatic Organisms

Table 5D-1. Lipid Content of Aquatic Organisms Used to Derive National Default Values of Lipid Fraction (f)

CSFII Consumption Category (Habitat)[a]	Common Name	Scientific Name	Species Mean Lipid Content (%)	CV[b]	No. Obs.	Data Source[c]	CSFII Mean Lipid (%)
Anchovy (estuarine)	Striped anchovy	*Anchoa hepsetus*	2.8	NR	23	1	6.1
	European anchovy	*Engraulis encrasicholus*	4.8	0.34	26	2	
	Northern anchovy	*Engraulis mordax*	10.7	NR	16	1	
Carp (freshwater)	Common carp	*Cyprinus carpio*	5.4	0.86	2,792	3	5.4
Catfish (freshwater)	White catfish	*Ameiurus catus*	4.3	0.58	204	3, 4, 5	2.9
	Black bullhead	*Ameiurus melas*	1.1	0.70	113	3, 4, 5	
	Yellow bullhead	*Ameiurus natalis*	1.4	0.99	95	3, 5	
	Brown bullhead	*Ameiurus nebulosus*	2.6	0.72	988	3, 4, 5	
	Channel catfish	*Ictalurus punctatus*	5.3	0.71	1,427	3, 4, 5	
Catfish (estuarine)	White catfish	*Ameiurus catus*	4.3	0.58	204	3, 4, 5	4.0
	Brown bullhead	*Ameiurus nebulosus*	2.6	0.72	988	3, 4, 5	
	Channel catfish	*Ictalurus punctatus*	5.3	0.71	1,427	3, 4, 5	
Cisco (freshwater)	Cisco	*Coregonus Artedii*	1.9	0.65	69	2	1.9
Clam (estuarine)	Hard shell clam	*Mercenaria mercenaria*	0.7	NR	47	1, 6	1.3
	Soft shell clam	*Mya arenaria*	1.2	NR	3	1	
	Venus clam (Littleneck Japanese)	*Tapes (venerupis) decussatus*	1.2	NR	15	1	
	Venus clam (Shortneck)	*Tapes japonica*	1.8	NR	3	1	
	Venus clam (Asari)	*Tapes philippinarum*	2.6		3	1	

ED_006490_00009970-00373

CSFII Consumption Category (Habitat)[a]	Common Name	Scientific Name	Species Mean Lipid Content (%)	CV[b]	No. Obs.	Data Source[c]	CSFII Mean Lipid (%)
	Venus clam	*Venus gallina*	0.9	NR	29	1	
	Venus clam (hard)	*Venus lusoria*	0.6	NR	5	1	
Crab (estuarine)	Blue crab	*Callinectes sapidus*	1.3	1.19	101	3	1.1
	Dungeness crab	*Cancer magister*	1.0	0.26	24	2	
	Queen crab	*Chionoectes opilio*	1.2	0.30	6	2	
Crayfish (freshwater)	Crayfish (mixed sp.)	*Astacus and Orconectes*	1.1	NR	5	2	1.1
Croaker (estuarine)	White croaker	*Genyonemus lineatus*	4.2	0.88	37	4, 5, 6, 7	3.0
	Atlantic croaker	*Micropogonias undulatus*	3.2	0.47	8	2	
	Yellowfin croaker	*Umbrina roncador*	1.8	0.70	3	5	
Eel (estuarine)	Eel, mixed species	*Anguilla spp.*	11.7	0.28	14	2	11.7
Flatfish (estuarine)	Sole and flounder	*Bothidae and Pleuronectidae*	1.2	0.80	596	2	1.2
Flounder (estuarine)	Sole and flounder	*Bothidae and Pleuronectidae*	1.2	0.80	596	2	1.2
Herring (estuarine)	Blueback herring	*Alosa aestivalis*	7.2	0.45	92	3	10.0
	Atlantic herring	*Clupea harengus*	9.0	0.51	2,524	2	
	Pacific herring	*Clupea pallasi*	13.9	0.39	128	2	
Mullet (freshwater)	Striped mullet	*Mugil cephalus*	3.8	0.62	43	2	3.8
Oyster (estuarine)	Pacific oyster	*Crassostrea gigas*	2.3	0.33	13	2	2.4
	Eastern oyster	*Crassostrea virginica*	2.5	0.56	193	2	
Perch (estuarine and freshwater)	White perch	*Morone americana*	3.5	0.72	682	3, 4	2.3
	Yellow perch	*Perca flavescens*	1.0	0.79	841	3, 5	
Pike (freshwater)	Northern pike	*Esox lucius*	0.6	1.01	904	3, 4	0.7
	Muskellunge	*Esox Masquinongy*	1.1	0.87	35	3	
	Chain pickerel	*Esox niger*	0.4	0.74	72	3, 4	

ED_006490_00009970-00374

CSFII Consumption Category (Habitat)[a]	Common Name	Scientific Name	Species Mean Lipid Content (%)	CV[b]	No. Obs.	Data Source[c]	CSFII Mean Lipid (%)
Rockfish (estuarine)	Striped bass	*Morone saxatilis*	5.3	0.59	7,657	3, 4, 5, 7	3.5
	Rockfish	*Sebastes spp.*	1.6	NR	81	2	
Salmon (estuarine & freshwater)	Pink salmon	*Oncorhynchus gorbuscha*	3.5	0.49	144	2	4.7
	Chum salmon	*Oncorhynchus keta*	3.8	0.62	13	2	
	Coho salmon	*Oncorhynchus kistuch*	2.9	0.75	617	3	
	Sockeye salmon	*Oncorhynchus nerka*	8.6	0.32	48	2	
	Chinook salmon	*Oncorhynchus tshawytscha*	3.4	0.93	873	3	
	Atlantic salmon	*Salmo salar*	6.3	0.74	7	2	
Scallop (estuarine)	Scallop, mixed species	*Pectinidae*	0.8	0.35	114	2	0.8
Shrimp (estuarine)	Shrimp, mixed species	*Panaeidae and Pandalidae*	1.7	0.39	100	2	1.7
Smelt, rainbow (estuarine & freshwater)	Rainbow smelt	*Osmerus mordax*	4.1	0.46	130	3, 8	4.1
Snails (freshwater)	Snails, mixed species	*Vivaparadidae, Helixidaed*	1.4	0.75	11	1	1.4
Sturgeon (estuarine & freshwater)	Lake sturgeon	*Acipenser fulvescens*	9.4	0.63	51	3	5.4
	White sturgeon	*Acipenser transmontanus*	1.3	0.67	7	4, 5, 7	
Trout, mixed spp. (freshwater)	Rainbow trout	*Oncorhynchus mykiss*	5.1	0.67	556	3, 4, 5	6.0
	Cutthroat trout	*Salmo clarki*	1.2	0.79	15	3	
	Brown trout	*Salmo trutta*	7.4	0.73	615	3, 4, 5	
	Brook trout	*Salvelinus fontinalis*	4.0	0.56	96	3, 4, 5	
	Lake trout	*Salvelinus namaycush*	12.3	0.62	910	3, 4, 5	
Trout, mixed spp. (estuarine)	Rainbow trout	*Oncorhynchus mykiss*	5.1	0.67	556	3, 4, 5	3.2

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CSFII Consumption Category (Habitat)[a]	Common Name	Scientific Name	Species Mean Lipid Content (%)	CV[b]	No. Obs.	Data Source[c]	CSFII Mean Lipid (%)
	Cutthroat trout	*Salmo clarki*	1.2	0.79	15	3	
Trout, (freshwater)e	Rainbow trout	*Oncorhynchus mykiss*	5.1	0.67	556	3, 4, 5	5.1
Whitefish	Whitefish, mixed spp.	*Coregonus spp.*	5.9	0.64	68	2	5.9

a Habitat designation (freshwater, estuarine) assigned to the CSFII consumption categories. See the Exposure Assessment volume of TSD Volume 2 (USEPA, 2003) for details.
b Coefficient of variation.
c Data sources: 1 = Sidwell (1981), 2 = Exler (1987), 3 = NSI (USEPA, 2001a), 4 = USEPA (1992a), 5 = CATSMP, 6 = primary literature, 7 = BPTCP, 8 = GBMB. See Section 6.2.2 for a description of data sources.
d In addition to these two families, specific genera represented include *Ampullaria, Vivaparus, Achatina, Murex, Thais, Nassa,* and *Aporrhais.*
e Information from the CSFII survey indicates that rainbow trout is appropriate for the "trout, freshwater" category.

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