
A Guide for the Use of Semipermeable Membrane Devices (SPMDs) as Samplers of Waterborne Hydrophobic Organic Contaminants

Regulatory and Scientific Affairs

PUBLICATION NUMBER 4690
MARCH 2002

A Guide for the Use of Semipermeable Membrane Devices (SPMDs) as Samplers of Waterborne Hydrophobic Organic Contaminants

PREPARED UNDER CONTRACT BY

James N. Huckins
Jimmie D. Petty
Harry F. Prest*
Randal C. Clark
David A. Alvarez
Carl E. Orazio
Jon A. Lebo
Walter L. Cranor
B. Thomas Johnson

Columbia Environmental Research Center
U.S. Geological Survey
USDI
4200 New Haven Road
Columbia, MO 65201

*California Analytical Division
Agilent Technologies, Inc.
1601 California Avenue
Palo Alto, CA 94303

MARCH 2002

ACKNOWLEDGMENTS

THE FOLLOWING PEOPLE ARE RECOGNIZED FOR THEIR CONTRIBUTIONS OF TIME AND EXPERTISE DURING THIS STUDY AND IN THE PREPARATION OF THIS REPORT:

API STAFF CONTACT

Thomas W. Purcell, Ph.D., Sr. Environmental Scientist

PAST AND CURRENT MEMBERS OF THE BIOMONITORING TASK FORCE

Dr. Raymon Arnold, Exxon Biomedical Sciences, Inc.

Marie Benkinney, Mobil Oil Corporation

Dr. Philip Dorn, Equilon Enterprise

Janis Farmer, BP PLC

Dr. William Gala, Chevron Texaco Company

Dr. Jerry Hall

Dr. Michael Harrass, BP Chemicals

Denise Jett, Phillips Petroleum Company

Dr. Eugene Mancini, ARCO

James O'Reilly, ExxonMobil Production Company

Dr. Lawrence Reitsema, Marathon Oil Company

Alexis E. Steen, ExxonMobil Corporation

Michael Swindoll, ExxonMobil Biomedical

Michael Tucker, Occidental Chemical Company

Carl Venzke, Citgo Petroleum Corporation

CONTRACTOR'S ACKNOWLEDGMENTS

WE THANK DR. JEFF WHYTE FOR HIS TECHNICAL ASSISTANCE IN THE COMPLETION OF THIS WORK. THE INSIGHTS OF DR. KEES BOOIJ, DR. ROBERT GALE AND DR. DON MACKAY ON THEORY AND MODELING ARE GREATLY APPRECIATED. WE ALSO ACKNOWLEDGE THE INTEREST AND SUPPORT OF SCOTT FOLWARKOW OF THE WESTERN STATES PETROLEUM ASSOCIATION, AND GARY RAUSINA OF THE CHEVRON RESEARCH AND TECHNOLOGY COMPANY. WE GIVE SPECIAL THANKS FOR THE GUIDANCE OF DR. WILLIAM GALA AND ALEXIS STEEN. FINALLY, WE DEDICATE THIS WORK TO THE MEMORY OF GARY RAUSINA.

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
EXECUTIVE SUMMARY	ES-1
1. INTRODUCTION.....	1-1
SYSTEM DESCRIPTION AND RATIONALE.....	1-4
2. SAMPLING OVERVIEW	2-1
APPLICABILITY OF SPMDs	2-1
METHOD SELECTION	2-3
Integrative	2-4
Equilibrium.....	2-5
COMMENTS ON AMBIENT CONCENTRATION EXTRAPOLATIONS	2-7
Accuracy of Water Concentration Estimates.....	2-7
SYNOPSIS OF PERFORMANCE RELATED VARIABLES.....	2-9
DEPLOYMENT	2-11
3. THEORY AND MODELING.....	3-1
MODELS FOR ESTIMATION OF WATER CONCENTRATION.....	3-3
Groundwater Concentration Estimation.....	3-6
Sediment Pore Water	3-7
SAMPLING APPROACH AND KINETICS.....	3-8
RATE CONTROL	3-11
FACTORS AFFECTING UPTAKE RATES	3-13
Sampler Design.....	3-14
Molecular Properties	3-14
Environmental Properties	3-16
CALIBRATION DATA	3-23
PERMEABILITY/PERFORMANCE REFERENCE COMPOUNDS (PRCS).....	3-31

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
4. STUDY CONSIDERATIONS	4-1
SOURCE AND PREPARATION OF SPMDS	4-1
Preparation and Evaluation of Materials	4-1
SPMD Assembly	4-2
SPECIFICATIONS OF THE STANDARD SPMD	4-3
STORAGE AND TRANSPORT	4-4
PRECAUTIONS/PROCEDURES DURING DEPLOYMENT AND RETRIEVAL	4-5
DEPLOYMENT METHOD	4-7
EXPOSURE DURATION AND ENVIRONMENTAL VARIABLES	4-8
5. SPMD ANALYTICAL CHEMISTRY	5-1
QUALITY CONTROL	5-1
SPMD-Fabrication Blanks	5-2
SPMD-Process Blanks	5-2
Reagent Blank	5-3
Field-Blank SPMDs	5-3
PRC Samples	5-4
SPMD Spikes	5-5
Procedural Spikes	5-5
Expected QC Results	5-6
PROCESSING AND RESIDUE ENRICHMENT	5-7
Potential SPMD Specific Interferences	5-10
INSTRUMENTAL ANALYSIS	5-12
DATA FORMAT AND COMPARABILITY	5-14

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
6. BIOASSAY OF SPMD EXTRACTS OR DILUENTS.....	6-1
MICROTOX AND MUTATOX	6-4
MFO-EROD	6-8
OVERVIEW OF ADDITIONAL ASSAYS	6-10
POTENTIAL INTERFERENCES.....	6-12
7. COMPARISONS TO BIOMONITORING ORGANISMS.....	7-1
8. CONCLUSIONS	8-1
REFERENCES	R-1
APPENDIX A	
SOURCES OF SPMD INFORMATION.....	A-1
APPENDIX B	
EXAMPLES OF APPLICATIONS	B-1
APPENDIX C	
ADDITIONAL CALIBRATION DATA.....	C-1
APPENDIX D	
WATER CONCENTRATION EXTRAPOLATION.....	D-1

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1-1	A Standard Lipid-Containing SPMD	1-3
1-2	Exploded Views of the Nonporous Membrane Size Exclusion Phenomenon.....	1-6
2-1	Plot of the Three Phases of SPMD Uptake.....	2-6
3-1	Profile of an SPMD Showing Potential Barriers to Analyte Mass Transfer or Uptake.....	3-13
3-2	Linear Regression Analysis of the Biofouling Mediated Percent Reduction (% _b) in Native PAH Uptake, as a Function of Log K _{ow}	3-19
3-3	Effect of Temperature and Log K _{ow} on PP PAH R _s Values	3-22
3-4	Illustration of Why the Permeability Reference Compound (PRC) Approach can be used to Adjust SPMD Sampling Rates.....	3-31
4-1	For Storage and Shipping, SPMDs are Placed in a Clean Metal Can.....	4-5
4-2	A Commercially Available Stainless steel Deployment Apparatus with a Capacity of 5 Standard SPMDs.....	4-8
5-1	Key Aspects of the SPMDs Sampling and Analysis Process	5-9
5-2	Illustration of Potential Problems Associated with Lipid Normalization of SPMD and Biota Chemical Concentrations: Kinetics versus Equilibrium	5-16
6-1	Illustration of SPMD <i>in vitro</i> Bioassay and Immunoassay Protocol.....	6-5
6-2	Ethoxyresorufin O-deethylase (EROD) Induction in H4IIE Rat Hepatoma Cells Exposed to an SPMD Dialysate	6-11
7-1	Comparison of the Patterns of Organic Contaminant Uptake Rates (as Related to Log K _{ow} s) by SPMD and Across Fish Gills.....	7-3
7-2	Comparison of Alkylated PAHs Residence Time in SPMDs and Oysters (<i>Crassostrea gigas</i>)	7-8
B-1	GC-MS Comparison of Ion Chromatograms of Extracts from Mussels (<i>Mytilus edulis</i>) and SPMDs	B-4
B-2	Comparison of PAHs Detected by GC-PID in SPMDs and Clams (<i>Corbicula fuminea</i>)	B-5

LIST OF TABLES

<u>Table</u>	<u>Page</u>
3-1	Summary of SPMD PP PAH Sampling Rates, Exchange Coefficients, and Partition Coefficients Derived from a 10° C Freshwater Flow-Through Exposure and Static Exposures 3-25
3-2	Summary of SPMD PP PAH Sampling Rates, Exchange Coefficients, and Partition Coefficients Derived from a 18° C Freshwater Flow-Through Exposure and Static Exposures 3-26
3-3	Summary of SPMD PP PAH Sampling Rates, Exchange Coefficients, and Partition Coefficients Derived from a 26° C Freshwater Flow-Through Exposure and Static Exposures 3-27
3-4	Summary of SPMD Organochlorine Pesticides Sampling Rates Derived from a 10° C Freshwater Flow-Through Exposure 3-28
3-5	Summary of SPMD Organochlorine Pesticides Sampling Rates Derived from a 18° C Freshwater Flow-Through Exposure 3-29
3-6	Summary of SPMD Organochlorine Pesticides Sampling Rates Derived from a 26° C Freshwater Flow-Through Exposure 3-30
6-1	Toxicological Evaluation of Polyaromatic Hydrocarbons (PAHs) with Microtox Basic Test and Mutatox.....6-7
6-2	Use of Microtox and Mutatox to Determine the Toxicity of SPMD Concentrates 6-8
7-1	Comparison of the SPMD Membrane and Respiratory Lamellae (gills) of Fish.....7-5
7-2	Comparison of SPMD and Oyster (<i>Crassostrea gigas</i>) Exchange Kinetics for Selected PAHs 7-7
C-1	PCB Congener Uptake Rate Constants for SPMDs C-2
C-2	Whole SPMD (lipid plus membrane) Uptake Rates (11° C and 19° C) for PCDDs, PCDFs and PCBs C-6
C-3	Physicochemical Properties and Standard SPMD (Whole Device) Uptake Kinetic Parameters (10° C) for Selected Insecticide.....C-7

LIST OF TABLES

<u>Table</u>		<u>Page</u>
D-1	Estimate of Water Concentration from SPMDs Exposed to 10° C Water for 21 Days Using the Excel® Calculator	D-5
D-2	Estimate of Water Concentration from SPMDs Exposed to 18° C Water for 21 Days Using the Excel® Calculator	D-9
D-3	Estimate of Water Concentration from SPMDs Exposed to 26° C Water for 60 Days Using the Excel® Calculator	D-13

ABBREVIATIONS AND ACRONYMS

A	Area (cm^2 or m^2) of an exchanging surface such as the SPMD membrane
Å	Angstrom, 10^{-10} meters
API	American Petroleum Institute
ACGIH	American Conference of Governmental and Industrial Hygienists
B	Biofilm or periphyton layer, lower case for subscript
BAF	Bioaccumulation factor (typically, concentration in whole tissue divided by concentration in water), includes both respiratory and dietary routes of chemical uptake
C	Chemical concentration in a medium, subscript specifies type of medium (C_w in this work is limited to residues dissolved in water)
cal	Calibration, i.e., laboratory generated data characterizing SPMD performance under controlled conditions
CERC	Columbia Environmental Research Center of the US Geological Survey
CF	Concentration factor in an SPMD, i.e., concentration in the whole SPMD divided by concentration in water
C.V.	Coefficient of variation, also called relative standard deviation, derived by dividing the sample mean into the standard deviation
D	Diffusion coefficient (cm^2 or m^2 / s or d)
DOC	Dissolved organic carbon, operationally defined as organic carbon that passes through a $0.2 \mu\text{m}$ or $0.45 \mu\text{m}$ filter
d	Day(s), or density
e	Subscript for exchange or loss (note that most subscripts denote the type of medium)
F	Flux; relative to Darcy's Law (F_w) the units are L or m^3/s or d , whereas relative to mass transfer of chemical across a barrier (F_c) the units are typically ng/s or d (also can be expressed as ng/d cm^2)
f	Field, i.e., derived from field exposures

EC₅₀	Toxicant concentration where 50% of test organisms exhibit adverse effects, i.e., effects concentration at the 50% level
ECD	Electron capture detector
EROD	Ethoxyresorufin O-deethylase, an enzyme, which is used as a bioindicator of planar aromatic contaminants
g	Gram
GC	Gas chromatography
GPC	Gel permeation chromatography, also referred to as size exclusion chromatography (SEC)
h	Hydraulic head, generally denoted as Δh and represents the difference in hydraulic head between the upper most point and the bottom of the strata or formation
I	Impedance to mass transfer or resistance
K	Equilibrium partition coefficient between two separate phases identified by subscripts, generally expressed as a dimensionless value
k	Rate constant (a unit mass specific coefficient, given in units of mL/d-g or 1/d) or mass transfer coefficient (cm or m/s), subscripts denote direction of chemical movement (rate constant) or type of medium (mass transfer coefficient)
L	Liter, or lipid
L_f	Length of a water bearing formation, used in groundwater modeling
LDPE	Low density polyethylene, layflat tubes with no additives used as SPMD membrane
LLE	Liquid-liquid extraction, the two phases must be largely immiscible
l	Effective thickness of a barrier (e.g., aqueous boundary layer) to the mass transfer of chemicals
M	Mass

m	Membrane or the LDPE tube used to make SPMDs
mol	Mole
n	Nano, one billionth of a mass unit.
MDL	Minimum detection limit for a specific chemical and method
MQL	Method quantitation limit for a specific chemical
MW	Molecular weight
OCs	Organochlorine pesticides
o	A subscript referring to “overall” or the lipid-like solvent “octanol”
P	Permeability coefficient, it is defined as $D_m K_{mw}$ for mass transfer in polymers with units of cm^2 or m^2/s or d , and is called hydraulic conductivity (i.e., P_{hc}) in groundwater modeling with units of velocity, i.e., cm or m/s or d
p	Pico, one trillionth of a mass unit
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
PAHs	Polycyclic Aromatic Hydrocarbon, a class of compounds largely derived by the production and use of fossil fuels
PP	Priority pollutant; a chemical or group of chemicals (e.g., the 16 PAHs that are emphasized in this work) listed by the US Environmental Protection Agency (EPA) as contaminants of concern
POC	Particulate organic carbon
PRC	Permeability (membrane control) or performance (aqueous boundary layer control) reference compound; defined as a noninterfering (analytically) compound spiked into SPMD lipid that is used to relate laboratory calibration data for SPMDs to actual <i>in situ</i> sampling rates or to develop <i>in situ</i> calibration data
QC	Quality control

R_s	Sampling or clearance rate which is applicable only to the linear uptake phase, not unit-mass or -volume specific, operationally defined as a standard 1-g triolein SPMD and given in L / d or mL / d
R²	Correlation coefficient, ratio of the sum of squares due to the regression equation divided by the sum of the squares about the mean
s	Second(s)
SA	Surface area
SEC	Size exclusion chromatography, used interchangeably with GPC in this work
SG	Silica gel, an adsorbent (normal phase) used for cleanup and fractionation of certain classes of chemicals
SOP	Standard operating procedure
SPE	Solid phase extraction
SPMD	Semipermeable membrane device, consists of a thin film of lipid, or another sequestration phase, sealed inside a LDPE tube of appropriate specifications
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEQ	Toxic equivalents to TCDD
TWA	Time weighted average, generally refers to the TWA chemical concentration during an exposure period
t	Time, given in units of “d” or “s”
u	Uptake, used as a subscript
USGS	United States Geological Survey
V	Volume (mL, L or m ³) of a matrix such as water, lipid and membrane
V_D	Lebas molar volume of a molecule
w	Water, used as a subscript

μ	Micron (10^{-6} m) relative to distance, and micro relative to mass (10^{-6} gram, i.e., microgram) and volume (10^{-6} L, i.e., microliter)
μ	Proportional to
μ_w	Viscosity of water at a specific temperature

ABSTRACT

Interest in the use of an *in situ* passive sampling approach for assessing environmental pollutant exposure has increased worldwide. A new paradigm for aquatic exposure assessments is emerging based on the use of lipid containing semipermeable membrane devices (SPMDs), which have been shown to be highly effective samplers of hydrophobic organic contaminants in water and air. The ability of SPMDs to concentrate trace (less than one-part-per-billion [$\mu\text{g/L}$]) concentrations of dissolved lipophilic residues to measurable levels is achieved by mimicking specific mechanisms of the aquatic bioconcentration (the uptake [concentration] of a substance by an organism from the surrounding medium [e.g., water], excluding the dietary route) process. The purpose of this document is to provide basic information and guidance on SPMD technology, and its appropriate use in aquatic systems. Emphasis is given to methods, applications, and theoretical issues related to the use of SPMDs for monitoring priority pollutant polycyclic aromatic hydrocarbons (PP PAHs), but other classes of hydrophobic organic contaminants are covered as well. This document includes key information on SPMD background, rationale, theory and modeling, technical considerations, supplier/source, chemical analysis and quality control, bioassay screening, comparability to biomonitors, examples of use, and sources of additional information. However, covering all potential environmental applications (e.g., vapor phase sampling) and relevant research results is beyond the scope of this work. Finally, use of this guide does not obviate the need for proper review and oversight procedures prior to the initiation of a project with SPMDs.

EXECUTIVE SUMMARY

BACKGROUND AND SPMD DEVELOPMENT

Environmental researchers have long recognized the tendency of some aquatic organisms to concentrate trace (less than one-part-per-billion [$\mu\text{g/L}$]) and ultra trace (less than one-part-per-trillion [ng/L]) residues of hydrophobic organic contaminants in water to relatively high levels (parts-per-million [mg/L]) in their fatty tissues. Because of the magnitude of this uptake process (bioconcentration), contaminants may reach harmful concentrations in aquatic organisms even when levels of bioavailable (chemicals that exist in a form that can be incorporated into tissues) residues in water are below the detection limits of many standard analytical methods. Unfortunately, attempts to improve and/or scale up standard sampling methods (e.g., liquid-liquid [LLE] and solid-phase extraction [SPE]) for this broad class of contaminants have often met with limited success, because of problems associated with the sampling and extraction of large volumes of water needed for acceptable detection limits. These and other limitations in analytical methods have often led to the use of biomonitoring organisms for assessing exposure of aquatic life to trace/ultra-trace levels of hydrophobic organic contaminants. However, organism-based sampling approaches also have inherent problems that can lead to a lack of proportionality between biomonitoring organism tissue concentrations and exposure concentrations. Since reasonably accurate exposure estimates are a fundamental element of the risk assessment process, new or improved methods for determining trace/ultra-trace levels of bioavailable hydrophobic organic contaminants are needed. In particular, methods are needed to estimate time-weighted-average (TWA) concentrations of hydrophobic contaminants in environments of concern.

Using a “mimetic chemistry” (i.e., use of processes in simple media to mimic more complex biological systems) approach, scientists at the US Geological Survey’s (USGS) Columbia Environmental Research Center (CERC) developed the lipid-containing

semipermeable membrane device (SPMD). Conceptually, lipid-containing SPMDs can be viewed as a bridge between analytical chemistry and biomonitoring methods. This passive *in situ* sampler simulates key aspects of the organic contaminant bioconcentration phenomenon using reproducible synthetic materials, without the uncertainty of estimating ambient environmental concentrations from biomonitoring organism tissues. The SPMD consists of a thin film of triolein (lipid) sealed inside a layflat thin-walled tube of nonporous (no fixed pores, only transient cavities) low-density polyethylene (LDPE). Previous research has shown that the diffusive transport of nonpolar organic contaminants through nonporous synthetic polymers like LDPE appears to approximate the movement of the same contaminants through more complex biomembranes. The transient pores in the SPMD LDPE tubing are about the same size (i.e., $\approx 10 \text{ \AA}$ diameter) as the postulated $9.8 \text{ \AA}$ size limit for gill membranes of fish. This solute size limitation allows only truly dissolved (water) or vapor phase (air) neutral organic contaminants, i.e., bioavailable residues not associated with particulates or dissolved organic carbon (water) macromolecules, to diffuse into the membrane and lipid. The lipid triolein (a neutral triglyceride) was chosen for use in SPMDs because it is a major storage fat found in many aquatic organisms and a close correlation has been shown between the equilibrium triolein-water partition coefficient (K_{TW}) and the octanol-water partition coefficient (K_{ow}) of hydrophobic organics. The lipid content of SPMDs is much higher than biomonitoring organisms and the membrane is also lipid-like. Thus, on an equivalent or unit mass basis, the SPMD has a much higher capacity for hydrophobic contaminants than organism tissues.

Because of the high lipid content of SPMDs, hydrophobic organic chemicals are integratively sampled under most exposure scenarios. Integrative sampling requires that the sampler act as an infinite sink for contaminant residues during the entire exposure period. Use of an *in situ* integrative sampling approach, provides a higher degree of assurance that episodic contaminant releases will be detected. The levels of detected contaminants are reflective of a time-weighted average (TWA) or cumulative dose of dissolved lipophilic chemicals. TWA values are widely used for monitoring personal

exposure to airborne chemicals in work environments, and the American Conference of Governmental and Industrial Hygienists (ACGIH) recommends the approach. However, until the advent of SPMDs, no passive sampling technology was available for aqueous environments that parallels the use of personal dosimeters for airborne chemicals in the work environment. SPMDs appear to sample nearly all nonionic hydrophobic chemicals (both vapor and dissolved phases) with molecular weights ≤ 600 Daltons. SPMDs have been shown to concentrate the following classes of chemical: PAHs, PCBs, OC pesticides, pyrethroid insecticides, alkylated phenols, chlorinated anisoles and veratroles, chlorinated dioxins and furans, chlorinated and brominated benzenes, certain heterocyclic aromatics, etc. Unfortunately, the advantage of an SPMD (i.e., the large volume of water cleared of bioavailable chemicals) over grab sampling or other relatively low volume techniques is generally diminished, when an analyte's $\log K_{ow}$ is < 3.0 . All the PP PAHs and their alkylated analogues have $\log K_{ow}$ s ≥ 3.0 and thus SPMDs are well suited for sampling PAHs.

MODELING

Earlier, we reported on the basic theory of SPMD operation and developed several mathematical models needed for estimating analyte concentrations in the ambient environment from SPMD levels. To reduce the complexity of the algorithm, the models in this original work focused on the SPMD lipid, and did not include the significant contribution of residues present in the membrane. SPMD extracts from the most widely employed method for analyte recovery (i.e., organic solvent dialysis of intact whole SPMDs) contain residues from both the SPMD lipid and membrane. Thus, the models reported in this guide are written to include analyte concentrations in the whole SPMD. We used a pseudo-two-compartment modeling approach (i.e., whole SPMD and water) in which the membrane is viewed as an extension of the lipid phase, and the membrane-lipid partition coefficient (K_{mL} or K_{mw}/K_{Lw} , where K_{mw} is the equilibrium membrane-water

partition coefficient) is used to adjust for the lower capacity (relative to lipid) of the membrane. For completeness, equations are included that permit estimation of ambient water concentration from all three phases of SPMD uptake (i.e., linear, curvilinear, and equilibrium). Also, equations are presented that permit determination of important model parameters such as the SPMD-water partition coefficient at equilibrium (i.e., the K_{SPMD}), estimation of the effects of analyte physicochemical properties and environmental conditions on SPMD sampling rates and the rate-limiting-step, and selection of the appropriate model(s) for estimation of analyte concentrations.

The focus of this work is the aquatic environment, which includes surface water, groundwater, and sediment pore water. Models are presented for estimating ambient concentrations of analytes for surface water and ground water, and environmental-specific limitations are discussed for all three-deployment scenarios. Unfortunately, we are unaware of any equation(s) to accurately relate SPMD levels to pore water concentrations.

Use of the mathematical models presented in “THEORY AND MODELING”, page 3-1, to estimate ambient TWA concentrations from SPMD levels requires calibration or sampling-rate data for analytes of interest. In Section 3 we include laboratory calibration data for PP PAHs and OC pesticides (note that calibration data for PCBs and other analytes are given in Appendix C, page C-1). However, successful application of laboratory-generated SPMD calibration data (e.g., sampling rates and K_{SPMD} values) to field deployed SPMDs depends on how closely laboratory- and field-exposure conditions match. To overcome this potential problem, we developed the permeability/performance reference compound (PRC) approach. PRCs are spiked into SPMD lipid prior to deployments. By comparing the amounts of PRC loss during field exposures and calibration studies, an environmental adjustment factor (EAF) can be derived, that permits estimation of actual *in situ* sampling rates. Using this approach to correct for a wide range of environmental conditions is expected to increase the accuracy of SPMD derived water concentration estimates.

SAMPLING AND ANALYSIS

Unlike most sampling methods, SPMDs are applicable to nearly all environmental conditions, selectively sample residues from the dissolved phase, operate *in situ*, and passively extract hydrophobic organic contaminants from relatively large volumes of water (typically > 10 L for a ≥ 10 d exposure to a standard SPMD [see “SPECIFICATIONS OF THE STANDARD SPMD”, page 4-3]). Also, hydrophobic contaminants are generally sampled without affecting the fractional distribution of residues in components of the sampled environment (excludes sediment-soil and some ground water), and fewer analyses (compared to grab samples) are required to assess the chronic exposure of aquatic organisms to contaminants.

One of the major advantages of SPMDs over biomonitoring organisms is that a standard design can be used regardless of water quality or environmental conditions. Samplers of uniform and reproducible material are required to delineate differences in sources (e.g., comparison of sample fingerprints or instrumental response profiles using pattern recognition programs) of complex chemical mixtures such as PAHs. In Section 4 we provide key specifications of the commercial or “standard” SPMD. Clearly, a standardized design is a prerequisite for global comparability of SPMD data.

Analytical interferences must be minimized in SPMDs to enable detection of trace/ultra-trace levels of hydrophobic contaminants. Because SPMDs sample organic vapors as well as dissolved residues, a clean room is required for the assembly of SPMD components. In addition, careful attention must be paid to adequate precleaning of SPMD materials. Storage and transport conditions must also be designed to minimize air exposure prior to the deployment and after the retrieval of SPMDs.

Quality control (QC) procedures used in the conduct of successful SPMD studies are similar to most analytical chemistry-based sampling approaches. QC samples should

address at a minimum, deployment, retrieval, storage, processing, fractionation, enrichment, and analysis. The exact level of QC required is determined during the development of the experimental design phase of a project and is dependent on project goals. In Section 4, we describe the various types of QC samples that may be used in a project using SPMDs. These include SPMD-fabrication blanks, SPMD-process blanks, reagent blanks, field-blank SPMDs, PRC samples, SPMD spikes, and procedural spikes. The QC samples listed above are designed to provide information on sample integrity and background interferences associated with the entire sampling and analytical process. In general, QC samples represent 20 to 50% of a sample set. Using the materials and procedures described in this document, the precision or coefficient of variation (C.V.) of replicate SPMD analyses is about the same or less than properly executed standard analytical methods (i.e., the C.V.s are typically $\leq 25\%$).

Other environmental sampling methods have QC samples similar to those listed for SPMDs. However, PRC samples are unique to the SPMD approach but are only used in projects that require estimation of ambient contaminant concentrations. As suggested earlier, PRC samples are designed to improve the accuracy of SPMD-derived water concentration estimates. PRCs should not be confused with analytical internal standards or surrogates, which are used to determine procedural recovery of analytes.

Processing, enrichment, and fractionation of analyte residues generally include the following steps: 1) removal of exterior surficial periphyton (organism that live attached to underwater surfaces) and debris, 2) organic solvent dialysis, 3) gel permeation chromatography (GPC), and 4) class-specific fractionation using Florisil[®], silica gel and/or alumina adsorption chromatography. These procedures are similar to those used for the analysis of fish tissues and sediments minus the sample drying and grinding steps. However, SPMD handling is less difficult and the mass of interferences (e.g., lipids) for an equivalent amount of sample is generally much less. Also, SPMD QC samples can usually be prepared with lower levels of analytical interferences than equivalent QC samples of

tissues and sediments. This allows the use of composite SPMD samples with a commensurate reduction in quantitation and detection limits. Analyte levels should be reported based on whole SPMD concentration, and lipid normalization should not be used. Potential errors in the format of SPMD data and in assumptions used in SPMD-biota comparisons are given in “DATA FORMAT AND COMPARABILITY”, page 5-14.

TOXICITY SCREENING

Because many toxic organic chemicals are in environmental waters at trace or ultra-trace levels, direct use of biomarker/immunoassay tests and acute bioassays may fail to show adverse effects or detect evidence of their presence. Unfortunately, this does not rule out the possibility of chronic toxic effects, especially when hydrophobic organic contaminants are highly bioconcentrated. SPMDs have been shown to enrich trace/ultra-trace residues that are known to bioconcentrate, thus they are often used as a convenient sample preconcentration method for the bioassays. SPMDs offer several advantages over other preconcentration methods. These include the following: 1) an *in situ* mimetic design, 2) only bioavailable dissolved residues are sampled, 3) sample extracts contain residues from episodic chemical releases during an exposure, and 4) a significant statistical advantage relative to biomonitors, due to high reproducibility. Some biomarkers and immunoassays are relatively inexpensive, when compared to the costs associated with instrumental analysis (e.g., mass spectrometry) of sample extracts. Thus, toxicity screening of SPMD extracts with selected biomarkers/immunoassays offers a convenient approach to prioritizing sample analysis.

In Section 6, we list (from the literature) the bioassays/immunoassay utilized in conjunction with SPMDs, and provide details of the use of Microtox®, Mutatox®, and EROD (ethoxyresorufin o-deethylase) induction to assess the toxicity of SPMD extracts. Also, we provide brief examples of several other types of bioassays applied to SPMD extracts. The

level of SPMD sample processing/cleanup differs for various assays, depending on the nature of the assay used and environmental exposure conditions. In some cases, the diluted lipid solution and the membrane extract have been used directly with no chromatographic enrichment steps (e.g., Microtox and Mutatox), whereas other assays have required both dialysis and GPC (e.g., EROD). Also possible chemical interferences are elucidated, which may necessitate additional cleanup of SPMD extracts prior to bioassay.

If an SPMD sample extract demonstrates a positive response to a biomarker assay relative to a reference site, questions often arise as to the relevance of the finding in regard to risk assessment. Clearly, the SPMD-biomarker/bioassay combination is a useful screening tool for ranking the potential toxicity of bioconcentratable residues at multiple sites and from multiple sources. Also, use of a preconcentration step such as SPMD sampling in conjunction with toxicity screening appears to be justified. This is because many trace organic compounds elicit toxic effects, only after being highly concentrated in organism tissues. Tissue residue concentrations resulting from exposure to persistent hydrophobic contaminants depends on the bioaccumulation factor (BAF). Unfortunately, the BAFs of hydrophobic organic contaminants often vary widely among different species and in some cases within a species. Also, depending on the specifics of the assay used, chemicals will be further concentrated during the cell line or organism incubation period. For the above reasons, it is very difficult to target an appropriate level of preconcentration by the SPMD for a particular assay. Obviously, the maximum level of preconcentration or the nonequilibrium SPMD concentration factor (CF, whole SPMD) should not exceed measured or estimated BCF of a chemical in the species of concern. Because of the aforementioned complications, the exact SPMD exposure duration and the target CF must be defined on a case by case basis.

RELATIONSHIP TO BIOMONITORING ORGANISMS

SPMDs have several advantages over biomonitoring organisms, when used to monitor trends in trace and ultra-trace hydrophobic organic contaminant concentrations. Unlike aquatic organisms, SPMDs can be used universally regardless of water quality and physical stressors, accumulated residues are not metabolized or actively depurated, most residues are integratively sampled (i.e., contaminants are accumulated without significant losses) over longer exposure periods, and SPMD concentrations have been shown to be proportional to ambient levels of dissolved chemical. Note that determination of contaminant sources, based on differences in instrumental profiles of sample residues (fingerprints), requires that concentrations of analytes in the sample matrices are proportional to actual environmental concentrations. Finally, most aquatic toxicity data bases are given in terms of dissolved residue concentrations, whereas residues accumulated in feral biomonitoring organisms typically represent both the dissolved phase and diet (i.e., the BAF).

The accumulation of hydrophobic organic compounds by SPMDs is expected to more closely mimic that of lipid-rich autotrophic (self-nourishing, photosynthetic or chemosynthetic) diatoms. Regardless of the type of aquatic organism that an SPMD best simulates, it is unreasonable to expect good correlations of chemical concentrations in SPMDs to all aquatic organisms. This is because reports in the literature clearly show that there are major differences in the accumulation rates of the same hydrophobic contaminant among species and test conditions. For example, existing bioaccumulation data suggests that a comparison of different species of biomonitoring organisms under similar exposure conditions would result in at least an order-of-magnitude difference in the relative amounts of the same chemical accumulated in their tissues. In the case of PAHs, differences in uptake rates across several species of test organism vary as much as two-orders-of-magnitude for the same compound. These differences mainly stem from inherent

interspecies variability in the volume of water cleared via respiration/feeding, lipid content and quality, and in xenobiotic metabolism and depuration rates.

Direct comparisons of bivalve mollusks (widely used as biomonitoring organisms because of the apparent inability of many mollusks to metabolize most contaminants) and finfishes to SPMDs have shown that in the case of nonionic hydrophobic organic contaminants, SPMDs accumulate a broader range of chemicals from water. This is not surprising, because SPMDs completely lack the active depuration mechanisms of living organisms and have higher lipid content. Even very hydrophobic chemicals, whose primary route of uptake by organisms is via diet-food chain (i.e., $\log K_{ow} \geq 6.0$), are concentrated to some degree by SPMDs.

In spite of the potential differences given above, comparisons of SPMD uptake rates (whole SPMDs) to that of bivalves and finfishes (whole body, wet weight) have shown that SPMDs concentrate PAHs and PCBs (polychlorinated biphenyl's) at a rate that ranges from about 0.2 to 5.0 times that of bivalves and finfishes. This similarity in SPMD and aquatic organism uptake rates for several groups of hydrophobic chemicals, suggests that the best correlation between SPMDs and organisms is expected at the level of chemical absorption and transport across the blood-water interface at the gills. We found that a plot of the uptake rates of PP PAHs by SPMDs has a parabolic shape similar to a plot of organic chemical uptake across trout gills. This comparison further substantiates that at the level of the blood-water barrier and SPMD membrane-water barrier in the uptake process, fish and SPMDs are similar. Also, the data suggest that the rate-limiting step in the uptake processes (i.e., aqueous boundary layer) may be similar for SPMDs and finfish. Based on the similarity of organism and SPMD rate constants, the magnitude of nonequilibrium CFs in fishes and SPMDs should be similar when both matrices are in the linear uptake kinetics phase. However, it is inappropriate to compare SPMD CFs with feral organism equilibrium BCFs (see "DATA FORMAT AND COMPARABILITY", page 5-14). Prediction of the equilibrium concentrations of contaminants in feral organisms from

SPMD levels requires estimates of water concentration (SPMD based) and the use of equilibrium partition models.

RESEARCH STATUS AND METHOD ACCEPTANCE

The use of SPMD technology for monitoring trace hydrophobic organic contaminants in environmental systems is growing rapidly throughout the world. With the information presented in this document, no major technical barriers exist to the reliable use of the technology for the determining the presence, transport/fate, and concentration of trace hydrophobic contaminants in aquatic environments. Analytical methods are now available to virtually eliminate interferences in the detection and quantitation of most analytes concentrated in SPMDs. Also, utilization of SPMDs in conjunction with biomarker tests for screening the toxicity of waterborne contaminants is growing. The use of SPMDs to preconcentrate trace environmental residues for biomarker tests appears to be justified, because of the need to account for the bioconcentration of compounds. However, the level of preconcentration is not well defined because of the inherent variability of organism BCFs. Side-by-side tests show surprising similarities in the uptake of hydrophobic organic contaminants by aquatic organisms and SPMDs. The closeness of these comparisons suggests that the same rate-limiting step controls the accumulation of hydrophobic organic contaminants by both SPMDs and biomonitoring organisms. Overall, few technical barriers appear to exist to the validation and general acceptances of SPMDs for monitoring hydrophobic organic contaminants in aquatic systems. Finally, SPMD data appear to be of equal or greater quality to other widely accepted screening tools used for environmental risk assessments of contaminants.

Section 1

INTRODUCTION

Information on the identity and TWA exposure concentrations of pollutants in environmental waters is a fundamental part of an ecological risk assessment process for chemical stressors. However, this type of data is often difficult to obtain because of limitations in conventional analytical and biomonitoring approaches. For example, data from widely used LLE and SPE methods provide information on water concentration only during the brief time of sample collection (i.e., grab sampling). Thus, detection of episodic events and estimation of more biologically relevant TWA values requires multiple LLE or SPE samples through time. Also, detection of trace- and ultra-trace- levels of bioconcentratable organic contaminants is problematic because standard LLE and SPE methods are designed for relatively small volumes of water (≤ 5 L). Even when large volume SPE samplers are used in conjunction with submersible pumping systems, major concerns exist with sample contamination, analyte losses, and procedurally mediated changes in the ambient distribution of target compounds due to the collection, filtration, and extraction of large volumes of water. Røe *et al.* (2000a) evaluated five methods for sampling PAHs in the North Sea. These methods included SPMDs, mussels, an *in situ* large volume SPE sampler, an SPE disk (Empore®), and LLE. In this evaluation, SPMDs and mussels were found to be most suitable approaches for monitoring PAHs in seawater.

Because bioaccumulation results in high concentration factors of many trace hydrophobic organic contaminants, biomonitoring organisms are widely used as environmental monitors. Typically this approach is used to determine the presence and distribution of contaminants, as well as aquatic organism exposure.

Problems/limitations related to the use of biomonitoring organisms include the lack of similar species at all study sites, the effects (lethality included) of non-contaminant related stressors on the accumulation of chemicals, and the metabolism and/or depuration of certain classes of bioavailable contaminants (Phillips, 1980). In addition, residues measured in tissues generally include the non-assimilated contents of the gut (may be a significant fraction of total) and attempts to depurate these materials can result in losses of tissue-incorporated residues. Finally, season, life stage, sex, and variations in lipid quality may affect concentrations of analyte residues in tissues.

In view of the problems associated with the use of the aforementioned contaminant assessment methods, considerable research on new approaches has been conducted. The success of personal passive monitors or dosimeters, in determining TWA or integrative (i.e., residues are retained despite any reduction in ambient levels) exposure concentrations of organic vapors in occupational environments (Fowler, 1982; ACGIH, 1990) has contributed to the application of the same principle to dissolved organic contaminants in aquatic environments. Early embodiments of this passive monitoring approach for aquatic environments (Södergren, 1987; Hassett *et al.*, 1989) consisted of organic solvent-filled containers or bags, with chemical uptake occurring across polymeric membranes. Although these devices had numerous shortcomings, their use in field studies (Södergren, 1987; Litten *et al.*, 1993) demonstrated that the *in situ* passive sampling approach had considerable potential. In an effort to optimize the solvent-containing passive sampler design, Zabik (1988) and Huckins (1988) evaluated the organic contaminant permeability and solvent compatibility of several candidate nonporous (i.e., free volume exists largely as transient cavities) polymeric membranes. The membranes included LDPE, polypropylene, polyvinyl chloride, polyacetate, and silicone or silastic. Criteria for membrane evaluation included solvent compatibility and environmental durability, analyte uptake rates, dialytic performance, cost, and convenience. Of the polymers tested, only LDPE and polypropylene were found acceptable.

Based on the aforementioned findings and using a “mimetic chemistry” approach to improve sampler design, scientist at the USGS’s CERC developed the lipid-containing SPMD (Huckins *et al.*, 1989; Huckins *et al.*, 1990a). The device is designed to mimic the biouptake of organic contaminants from water alone (bioconcentration process) and provide an integrative estimate of dissolved hydrophobic contaminant concentrations, without the many variables associated with the use of organisms (see “*SPMD Homepage*” URL on page A-11). Figure 1-1 is a picture of a commercially available standard SPMD. Although the device is simple in design, the mechanisms governing its performance are complex.

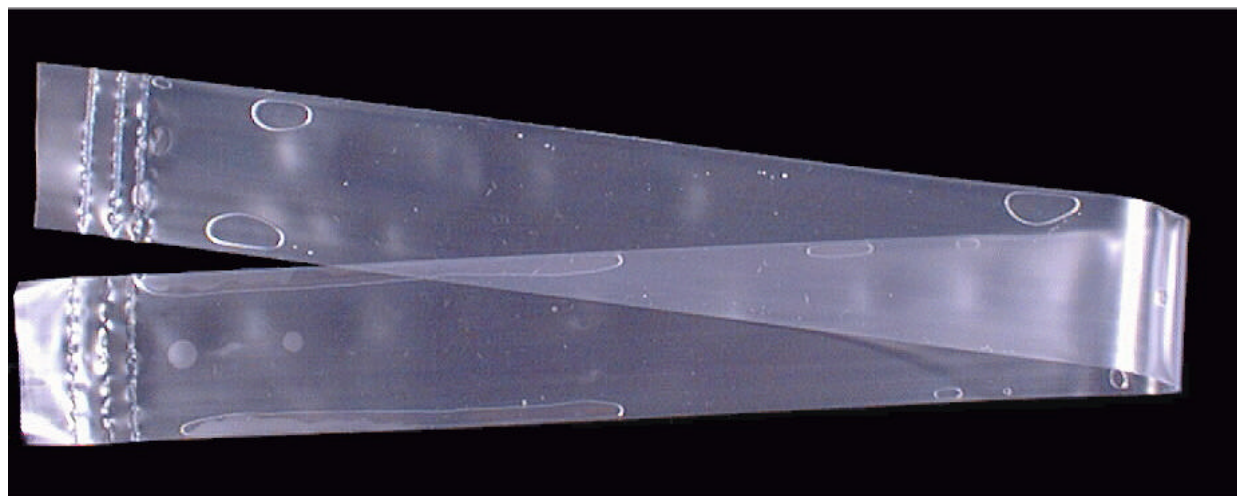


Figure 1-1. A standard lipid-containing SPMD with three molecular welds near each end. *Note the low interfacial tension causes intimate contact (i.e., the presence of a lipid film on the membrane interior surface) between the triolein and the membrane even where air bubbles exists.*

SYSTEM DESCRIPTION AND RATIONALE

An SPMD consists of a thin film of triolein sealed in a layflat thin walled tube of nonporous LDPE. The thin film (triolein) layflat design (Huckins *et al.*, 1990a) has the greatest surface-area-to-volume (SA/V) ratio of any *in situ* sampler configuration, with the exception of hollow fiber bundles and strips of thin LDPE film. On an equivalent-mass basis (e.g., g of whole SPMD versus g of whole body fish) the SA of an SPMD is more than 7-fold larger than the SA of a gill integument of a fish (Hayton and Barron, 1990). The direct dependency of SPMD sampling rates on surface area is shown in "THEORY AND MODELING", page 3-1. Triolein or 1,2,3-tri-*cis*-9-octadecenoyl glycerol used in SPMDs is a major neutral triglyceride found in most organisms. Although fish lipid (Huckins *et al.*, 1990a) and silicone fluids (Petty and Orazio, 1996; Petty *et al.*, 1997) have been successfully used as SPMD liquid phases, triolein has several advantages. Its attributes include the availability as a high purity synthetic product, a low transition temperature from a liquid to a wax (i.e., melting point of 0 °C) permitting use at low temperatures, and a large capacity to solubilize nonpolar organics. Also, a close correlation (Chiou, 1985) exists between the equilibrium triolein-water partition coefficient (K_{LW}) and the K_{OW} of hydrophobic organics, and it has a low LDPE permeability, even during dialytic recovery of analytes from SPMDs (Huckins *et al.*, 1990b; Meadows *et al.*, 1993). In addition, triolein provides a convenient reservoir for permeability or performance reference compounds (PRCs). For more information about PRCs see page 3-31.

As indicated earlier, the selection of nonporous LDPE layflat tubing for SPMDs was in part based on stability in organic solvents (required for dialysis and membrane cleaning) and optimal membrane permeability of analytes (both uptake and dialytic recovery phases). Polymeric film (LDPE) dialysis in organic solvent has been demonstrated to be a highly effective method for separating organic contaminants from sample lipids (Huckins *et al.*, 1990b; Meadows *et al.*, 1993; Meadows *et al.*, 1996; Bergqvist *et al.*, 1998a; Strandberg

et al., 1998). Thin-walled layflat LDPE is widely available and, because it is a thermoplastic, molecular welds (heat seals) can be used to enclose the lipid phase.

The underlying principle of solute size discrimination in the uptake and loss of chemicals by SPMDs is shown in Figure 1-2, page 1-6. In nonporous polymers such as LDPE, free volume is formed by random thermal motion of polymer chains, and the volume associated with “fixed pores,” which exist only in the crystalline regions of the polymer, is largely insignificant (Comyn, 1985). Thus, sampled molecules essentially dissolve into the polymer (Comyn, 1985). The diameters of the transient polymeric cavities range up to ≈ 10 Å (Hwang and Kammermeyer, 1984), which precludes sampling of the waterborne residues associated with particulate organic carbon (POC) or dissolved organic carbon (DOC) such as humic acids. Also, it is noteworthy that the postulated size of transient cavities in biomembranes is 9.8 Å (Opperhuizen *et al.*, 1985). The molecular size limitation of nonporous polymers suggests that only dissolved chemicals (molecular weights < 600 Daltons) will be sampled by SPMDs, which has been corroborated by the work of Ellis *et al.* (1995). However, in some environments contact of the membrane surface with oil droplets, DOC, etc., could result in direct absorption of chemicals. Ions of organic and inorganic chemicals are not sampled by SPMDs, because charged species are hydrophilic and are essentially insoluble in nonpolar LDPE (Roff *et al.*, 1971). Water quality variables, such as pH and salinity (Huckins *et al.*, 1999), may affect the concentrations of dissolved residues in environmental waters but should have no effect on SPMD sampling rate (i.e., daily volume of water cleared of chemical, exhibits first-order kinetics). This type of sampling process can be conceptualized as a constant volume of water cleared or extracted of chemical by an SPMD per unit time. Although the volume of water sampled is independent of concentration, the amount of an analyte extracted is proportional to water concentration.

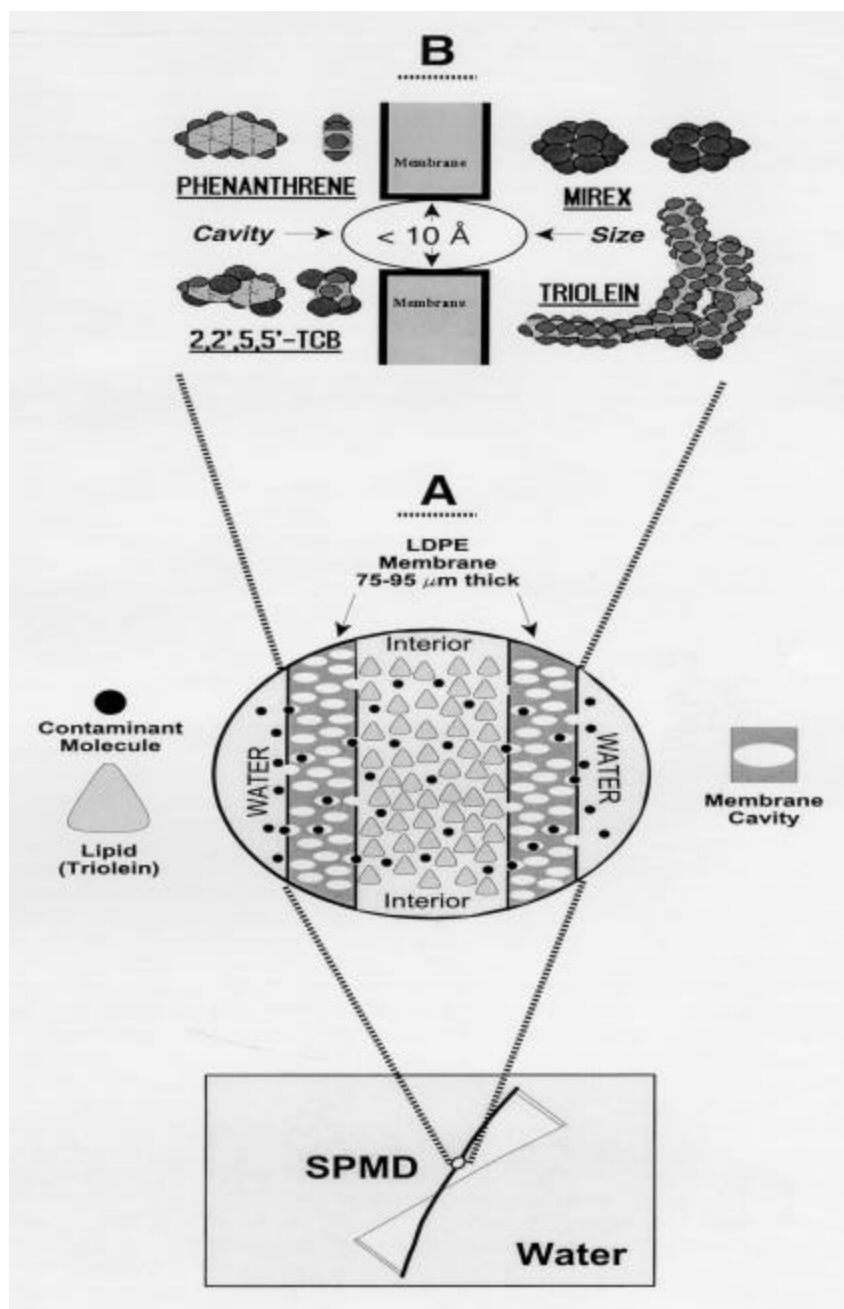


Figure 1-2. Exploded views showing the nonporous membrane size-exclusion phenomenon in the uptake and loss of organic compounds. *Illustration “A” shows the movement of contaminant molecules through transient pores in the membrane and retention (membrane exclusion) of much larger lipid molecules. Illustration “B” shows similarly scaled space-filled molecular models of some organic contaminants and triolein, along with the hypothetical polymer pore (transient) size.*

Because SPMDs operate passively, are used *in situ*, and extract a relatively small volume of contiguous water per unit time, it is unlikely that sampling causes significant changes in ambient concentrations or phase distributions of chemicals in surface waters. Using SPMDs to sample bioavailable residues associated with sediment, soil, and groundwater wells may be exceptions to the above statement, due to the slow desorption rates of some chemicals from the organic carbon of sediment-soil particles and nearly stagnant conditions (e.g., some monitoring wells are located in areas with very low hydraulic conductivities or exchange rates). In exposure scenarios, where compound desorption is rate limiting, reduced concentrations of dissolved or vapor phase analytes would be expected at the membrane surface. With these possible exceptions, SPMD derived values of ambient concentrations appear to be reasonably accurate (see "COMMENTS ON AMBIENT CONCENTRATION EXTRAPOLATIONS", page 2-7). Also, use of PRCs in SPMD lipid, as proposed by Huckins *et al.* (1993) should further improve the accuracy of water concentration estimates.

In most environments, the fractional amount of hydrophobic chemicals in the dissolved phase at any moment in time is generally small. However, for compounds with $\log K_{ow} < 6.0$, this fraction is the major source of residues accumulated in fish tissues (Connell, 1990). Note that dissolved hydrophobic residues are readily bioavailable and exchangeable, and are replaced by mixing and desorption from POC and DOC. Due to the processes of bioaccumulation and biomagnification, dissolved chemicals are often toxicologically significant even at ultra-trace levels. For compounds with $\log K_{ow} > 6.5$ (Connell, 1990), diet represents the primary route of biouptake for many higher trophic level organisms. However, regardless of the route of residue uptake, significant differences in the presence and concentrations of chemicals in fish tissues and SPMDs are often observed because most aquatic organisms metabolize and actively depurate many contaminants, especially polycyclic aromatic hydrocarbons (PAHs). SPMDs generally provide TWA concentrations of dissolved contaminants over the entire exposure period,

which is not always the case for aquatic organisms. See additional information on this subject in “COMPARISONS TO BIOMONITORING ORGANISMS”, page 7-1.

Conceptually, SPMD data fill a gap between exposure assessments based on direct measurement of total residues in water and the analysis of residues present in tissues of feral or biomonitoring organisms. Unlike most widely used analytical methods, residues in SPMD extracts are only representative of the readily bioavailable or dissolved phase, which is important regardless of the dominant route of biouptake (i.e., water or diet). Also, most aquatic toxicity data in the literature are based on nominal chemical concentration in the dissolved fraction. Thus, SPMD-derived estimates of the water concentrations of chemicals of known toxicity are directly applicable to the environmental risk assessment process.

In this report, we provide basic information and guidelines for the use of SPMDs in water. This information includes theory and modeling, applicability and project considerations, analytical chemistry, bioassay of SPMD concentrates, the comparability of SPMDs to biomonitoring organisms, and appendices that include sources of additional information, calibration data, a computer model for water concentration estimation, and examples of applications. Please see the “TABLE OF CONTENTS” for the location of subsections with more specific information.

Section 2

SAMPLING OVERVIEW

APPLICABILITY OF SPMDS

Perhaps the first question that should be addressed before employing SPMD technology is the suitability of the approach for the chemicals of concern to the investigator. Standard SPMDs are designed to concentrate hydrophobic chemicals such as PAHs, PCBs, organochlorine pesticides (OCs), etc. Hydrophobic compounds are characterized by a lack of polar functional groups and a very low potential for ionization at environmental pHs (i.e., a range of about 4.5 to 9.0). Nearly all hydrophobic (i.e., chemicals with $\log K_{ow} \geq 3.0$) neutral organic compounds will be significantly concentrated above ambient levels. However, at $\log K_{ow} < 3.0$, the advantages of the SPMD are often not significant relative to other sampling procedures. See "SAMPLING APPROACH AND KINETICS", page 3-8, for discussions on the use of K_{ow} s to estimate the capacity of SPMDs for chemicals. SPMDs are not applicable to ionized chemicals, such as free metals, and large organic molecules (molecular weight > 600 Daltons and/or cross sectional diameter > 10.5 Å). Note that for compounds such as chlorinated phenols with pK_a s < 9.0, the environmental pH determines the ratio of ionized to neutral species, and thus directly impacts the amount of chemical sampled.

From the literature, it is apparent that SPMDs sample a wide variety of dissolved (bioavailable) hydrophobic contaminants in aquatic systems. Examples of chemicals classes shown to concentrate in SPMDs include, but are not limited to the following:

PAHs	Pyrethroids
PCBs	Alkylated phenols (e.g., nonyl phenol)
OCs	Moderate to hydrophobic organophosphate pesticides
PCDDs	Non-ionic organometallic chemicals
PCDFs	Certain heterocyclic aromatics

Again, numerous hydrophobic organic compounds are not listed above that may be satisfactorily sampled by SPMDs.

The second question that should be addressed before choosing SPMDs for a project is the data quality requirements. Two extreme levels are litigation quality data (i.e., legally admissible) and screening data (note that rigorous quality control can be applied to screening tests). The SPMD approach can be readily used in screening projects, such as the presence/absence, sources, and relative amounts of chemicals (ranking) measured in SPMDs at different sites, to more in-depth studies designed to estimate the ambient concentrations of chemicals. For projects requiring litigation quality data, study results are typically generated by EPA or industry standard methods in conjunction with a formal set of quality control and assurance (QA/QC) guidelines/parameters. Particular attention must be made to security issues (QA) such as sample chain of custody. Because EPA and industry standard methods are often more than a decade behind the best available technology, there has been increased use of current, but well-established, nonstandard methods in litigation. Although the SPMD approach is widely used by environmental investigators and is beginning to gain acceptance from regulatory and resource management agencies (e.g., the states of Virginia and Wisconsin, and certain EPA regions), the authors are not aware of any studies conducted with protocols adequate for litigation. The SPMD studies presented herein may meet the criteria for QC but fail to meet the QA requirements for litigation, such as chain of custody documentation. However, as *a priori* acceptance of SPMD technology becomes more widespread, and studies are conducted with more stringent QA standards, the likelihood of the successful use of SPMD data in litigation will increase.

Another applicability issue relates to the type of water sampled. Waterborne hydrophobic contaminants are present in surface water, groundwater, and sediment pore water. SPMDs readily sample dissolved chemicals in all three matrices but the ability to extrapolate from analyte concentrations in the device to ambient water concentrations

differs significantly. See "MODELS FOR ESTIMATION OF WATER CONCENTRATION", page 3-3, for more details.

The appropriateness of using SPMD data to predict equilibrium concentrations of bioconcentratable contaminants in aquatic organisms is dependent on the level of accuracy required. Obviously, SPMDs do not account for dietary uptake and trophic transfer (biomagnification), which can cause residue concentrations in tissues to exceed equilibrium levels (Connolly and Pedersen, 1988) in some predators. However, for compounds with $\log K_{ow} \leq 6.0$, SPMDs provide reasonable estimates of chemical exposure, i.e., TWA concentration. TWA exposure data is critical to the successful use of bioconcentration models for the prediction of contaminant tissue concentrations.

SPMD technology has been used for the following applications in aquatic environments: 1) determination of the presence, sources, and the transport/fate of hydrophobic organic pollutants, 2) estimation of ambient TWA dissolved or vapor phase chemical concentrations, 3) estimation of the fluxes of bioavailable (i.e., dissolved phase) chemicals in aquatic systems, 4) *in situ* mimetic concentration of bioavailable chemicals for bioassay/biomarker tests and immunoassay, and 5) estimation of organism waterborne exposure. Details of most of these applications are covered in subsequent sections. Also, see "Appendix A", page A-1, for examples of most of the above applications. However, there are too many good examples of application "1)" and "3)" to include them in APPENDIX B. For example, the works of Petty *et al.* (1998) and Bergqvist *et al.* (1998b) should be examined by investigators interested in applications "1)" and "3)".

METHOD SELECTION

Two approaches are typically used to estimate ambient chemical levels from concentrations in SPMDs. One is based on integrative sampling or a TWA approach and the other is based on the attainment or close approach of equilibrium. Obviously, a

third possibility exists in which an analyte is sampled in both the linear and the curvilinear phases of uptake. This possibility is discussed separately in “THEORY AND MODELING”, page 3-1. Depending on the suite of chemicals present at a sample site, environmental conditions, and the duration of the exposure, analytes sequestered by an SPMD may be in the linear uptake (integrative sampling), curvilinear, and equilibrium partitioning phases of sampling (Figure 2-1, page 2-6). Selection of the appropriate SPMD design and exposure duration is predicated by the choice of sampling approach (integrative versus equilibrium) used. Before selecting a sampling approach for the analytes of interest, a better understanding of the relationship between sampler exchange kinetics and equilibrium capacity is in order.

The rate of chemical loss from an SPMD relates to both the unit capacity (i.e., the K_{SPMD}) of the device and the magnitude of the uptake rate constant (Huckins *et al.*, 1993). By assuming that SPMD exchange rates obey first-order kinetics (shown by Huckins *et al.*, 1993) and that the SPMD capacity is set (i.e., constant K_{SPMD} , same materials used and temperature is constant), a rapid rise to equilibrium concentration means a rapid loss (i.e., proportional increase in loss rate) when reductions in ambient chemical concentrations occur. This relationship is given by (Huckins *et al.*, 1997a)

$$k_e = k_u / K_{SPMD} \quad \text{(Equation 2-1)}$$

and rearranging

$$K_{SPMD} = k_u / k_e \quad \text{(Equation 2-2)}$$

Where k_u is the uptake rate constant and k_e is the clearance rate constant. Again, remember that K_{SPMD} can be viewed as the sampler unit capacity for a specific analyte.

Integrative

This approach provides an estimate of the cumulative dose (TWA) of contaminants during a specified exposure period. Unlike samplers that rapidly achieve equilibrium

(characterized by high loss rates and low capacity), hydrophobic residues from episodic chemical events during the initial part of an exposure are retained in SPMDs. Thus, integrative sampling is possible for a chemical due to a vanishingly small rate constant for residue loss (Equation 2-1). However, integrative sampling occurs only during the linear phase of SPMD uptake. As suggested earlier, the associated rate constant (k_u) and sampling rate (R_s) are independent of environmental concentration. Using standard SPMDs (see "SPECIFICATIONS OF THE STANDARD SPMD", page 4-3, for specifics), compounds with moderate to very large K_{LW} values (i.e., $> 10^5$) are integratively sampled in quiescent environments during exposures of one month or less. For compounds with $K_{ow} < 10^5$ (e.g., naphthalene $K_{ow} = 2.2 \times 10^3$), Petty *et al.* (1997) and Huckins *et al.* (1995a) found that integrative sampling was achieved for extended periods by dispersing small amounts of adsorbent in the SPMD triolein. Because uptake rates are affected by temperature, biofouling and turbulence-flow velocity (compounds with $\log K_{ow} > 4.5$), PRCs should be used when exposure and laboratory calibration conditions are dissimilar.

Equilibrium

The equilibrium partitioning approach has been widely used to model chemical concentrations in environmental media, largely because of its simplicity. A potential advantage in using the equilibrium partition approach for prediction of ambient chemical concentration is that the effects of biofouling, turbulence-flow velocity, and temperature on sampling rates can be ignored in some cases. However, these environmental variables also affect the time to equilibrium (see "Equations 3-18 and 3-20" in next section) and temperature may affect the magnitude of the equilibrium partition coefficient (i.e., K_{SPMD}). Thus, it is incumbent on the investigator to demonstrate that steady state has been achieved, which requires the use of PRCs. When measuring sampler concentrations through time, care must be used not to misinterpret a near-plateau in sampler concentrations due to falling water concentrations and/or biofouling, as the approach to equilibrium through time.

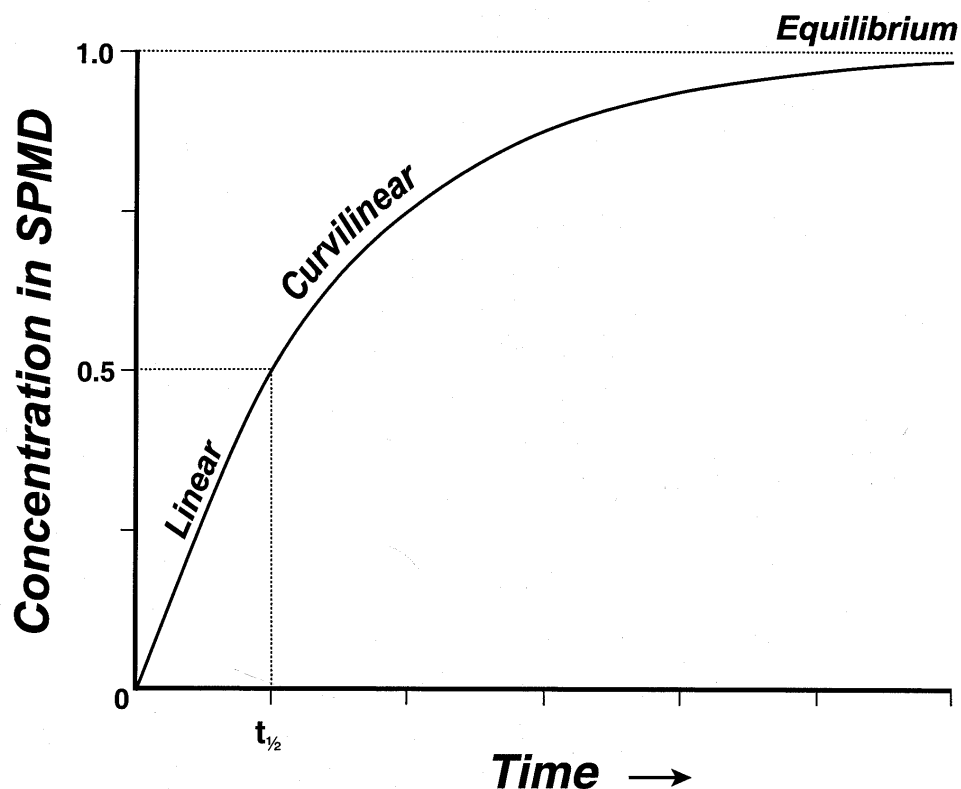


Figure 2-1. Plot of the Three Phases of SPMD Uptake. *The amount of time that a chemical remains in the linear uptake phase or takes to reach equilibrium is dependant on molecular properties of the analyte, environmental conditions, and SPMD design. The time axis is given in half-times ($t_{1/2}$) to equilibrium.*

To increase the likelihood that compounds with high K_{ow} s reach equilibrium during exposure periods of 30 days, the capacity of equilibrium partition samplers to retain chemicals with low or intermediate level $\log K_{ow}$ s (≤ 4.5) will necessarily be low. In this case, a scenario can be envisioned in which an episodic contaminant event occurs early in an exposure and by the end of the exposure, residue concentrations in the device falls below the detection limit. On the other hand, an episodic event occurring in the latter part of the exposure would result in ambient water concentration estimates that are much

higher than actual TWAs during the course of the study. Because of the potential for significant errors in environments with variable water concentrations, the equilibrium partition approach should be viewed as a reliable indicator of the presence of a chemical, but not always the absence (potential false-negative error).

COMMENTS ON AMBIENT CONCENTRATION EXTRAPOLATIONS

The use of the kinetics models described herein for estimating TWA ambient concentrations of dissolved residues generally requires SPMD calibration data. These rate constants and partition coefficients (i.e., k_u , k_e , R_s [k_u times SPMD mass in grams], K_{SPMD} , K_{mw} , and K_{Lw}) are measured under controlled laboratory conditions, *in situ* via PRC loss rates, and/or derived from quantitative-structure-activity-relationships. Note that in the main body of this guide calibration data are reported for priority pollutant PAHs and organochlorine pesticides. Also, in appendix B, limited calibration data are given for PCBs, chlorinated dioxins and furans, and selected insecticides. The key to the successful application of a kinetic modeling approach for the estimation of water concentrations is in ensuring that separately measured or derived calibration data accurately reflect actual values during environmental exposures. To achieve this goal, calibration conditions (e.g., temperature, flow/turbulence, and biofouling) must approximate environmental exposure conditions or permeability/performance reference compounds (PRCs, see subsequent discussions on this subject) must be used to correct for any differences between conditions of calibration and exposure or to generate *in situ* calibration data.

Accuracy of Water Concentration Estimates

Comparisons of SPMD water concentration estimates to water concentration data obtained by other analytical approaches is not straightforward, because no other methods exclusively sample dissolved phase residues. However the results of the comparisons

that have been made are generally encouraging. In the laboratory, Huckins *et al.* (1993) compared SPMD model estimates of phenanthrene and 2,2',5,5'-tertachlorobiphenyl water concentrations, derived from SPMD levels, to the concentrations of the same analytes determined using a traditional LLE method. For both analytes estimated (SPMD) and measured (LLE) values varied by only 1.5 or less. However, the focus of this work was to test the accuracy of the exponential model used for water concentration estimates and not the SPMD method. Ellis *et al.* (1995) centrifuged (flow through system), and filtered water samples (Upper Mississippi River) through a tangential-flow ultrafilter that removed colloids and DOC with cross-sectional diameters $> 50 \text{ \AA}$. SPMDs were deployed at the same site and water concentration estimates of OC pesticides were compared to results from the analysis (LLE and GC-ECD) of the ultrafilter permeates. The ratios of OC pesticide (those detected by both methods) concentrations derived from SPMDs and measured in ultrafilter permeates were 1.0 ± 0.4 for lindane, 1.6 ± 0.8 for dieldrin, and 1.9 ± 1.3 for pentachloroanisole. Clearly, these values are remarkably close, in view of the number of variables involved in comparing the two methods. Rantalainen *et al.* (1998) compared the water concentrations (Lower Fraser River, Canada) of a number of chlorinated industrial contaminants derived from SPMDs to those obtained by using an Infiltrex[®] water sampler and found that the results were similar in magnitude (i.e., differences were generally much less than an order-of-magnitude). Recently, Huckins *et al.* (2000) used SPMDs to estimate groundwater concentrations of OC pesticides and found 69 ng/L of dieldrin (more than 100 fold higher than the other OC pesticides) in one well. Earlier, a grab sample from the same well found dieldrin concentrations of 110 ng/L (Eidelberg, 1998). The results of these studies suggest that the accuracy of SPMD-derived water concentration estimates is quite good, considering the number of variables involved in comparisons and the unique specificity of the approach.

SYNOPSIS OF PERFORMANCE RELATED VARIABLES

Knowledge of the barrier or layer with the largest resistance to mass transfer in the chemical uptake process is important, because it can affect how the sampler responds (i.e., *in situ* sampling rates) to different environmental conditions and differences in the lipophilicity (i.e., K_{ow}) of a mixture of analytes. There are potentially three rate-limiting barriers to the uptake of aqueous residues by environmentally exposed SPMDs. The barriers include the so-called aqueous boundary or diffusion layer (a hydrodynamically complex region at the membrane-water interface with resistance to mass transfer), periphyton and debris (e.g., particulate organic carbon) or a biofilm on the exterior membrane surface, and the membrane. When multiple barriers exist, mass transfer theory assumes that resistance is additive and independent. Also, the resistance in a particular region/layer is directly proportional to the thickness of the barrier. If the affinity of a diffusant molecule for a barrier phase is greater than that of water (reflected by the magnitude of the partition coefficient), resistance is reduced in proportion to the magnitude of the appropriate partition coefficient (e.g., K_{mw}). Thus, rate control is dependent on both environmental conditions (e.g., thickness of the aqueous diffusion layer and biofilm) and physicochemical properties of the chemical of interest.

For compounds under membrane control, boundary layer thickness or water flow rates/turbulence has little effect on SPMD sampling rates. However, structural features of analyte molecules are expected to be more important because of specific polymer-solute interactions. In the case of chemicals under boundary layer control, water flow rates/turbulence can significantly affect SPMD sampling rates. In environmental systems the effective thickness of the aqueous boundary layer can vary from about 10 μm (extremely turbulent/high flow conditions) to 1,000 μm (deep stratified lakes or deep seas/oceans). Calibration data given in this guide (see "CALIBRATION DATA", page 3-23) were generated under conditions of relatively low flow (i.e., $< 1 \text{ cm/sec}$) and the boundary layer thickness is estimated at 200-400 μm . Under these flow conditions; rate control appears to switch from the SPMD membrane to the aqueous boundary layer when $\log K_{ow}$ s of analytes exceeds 4.5. Other investigators (Booij *et al.*, 1998) have shown that

an increase in flow velocity (at the SPMD membrane surface) of 1 cm/s to 30 cm/s. resulted in about a 4-fold rise in the sampling rates of diffusion layer controlled chemicals. Thus, the effects of major differences in boundary layer thickness on SPMD sampling rates are significant, but can be reduced by using care in site selection and by the design of the deployment apparatus.

Biofouling of SPMDs results in an additional barrier to the mass transfer or uptake of analyte residues. The thickness and coverage of periphyton on the membrane surface typically vary from site to site, but also can vary among replicates. Long exposures (> 28 d) of SPMDs in biologically rich ecosystems can result in heavy biofouling, where coverage is near complete and biofilm thickness approaches or exceeds 1 mm. In side-by-side laboratory exposures of non-fouled and heavily fouled SPMDs to native and perdeuterated PP PAHs, we found that the impedance to residue uptake was dependent on the magnitude of analyte K_{ow} (Huckins *et al.*, 1994). This investigation demonstrated that a four-orders-of-magnitude increase in the log K_{ow} values (3.0 to 7.0) of PP PAHs resulted in the decline of PAH sampling rates ($R_{s,s}$) by 20 to 70%. These data are consistent with theory, which suggests that resistance to mass transfer through a polar proteinaceous biofilm will rise as diffusant molecules become increasingly large and nonpolar. Obviously, when sampling rates are reduced by > 50%, rate control switches to the biofilm. For additional details, see "FACTORS AFFECTING UPTAKE RATES", page 3-13.

In general the effects of temperature on SPMD sampling rates are complex, appear to be chemical class specific, and are dependent on which barrier controls the rate of uptake. However, higher environmental exposure temperature generally results in an increase in SPMD sampling rates. For priority pollutant PAHs, a 16 °C range in exposures temperatures (i.e., 10 to 26 °C) changed SPMD sampling rates by less than two fold. For organochlorine pesticide exposures (similar experimental conditions), sampling rates increased by as much as four fold. See "FACTORS AFFECTING UPTAKE RATES", page 3-13 for additional details.

Because the aforementioned variables may significantly change measured SPMD sampling rates during exposures, relative to those measured in calibration studies, we proposed the use of PRCs to permit estimation of actual exposure R_s values. PRCs are non-interfering (analytically) compounds, such as perdeuterated (all hydrogen atoms replaced by deuterium atoms) PAHs with moderate to fairly high fugacity (escaping tendency), added to SPMD triolein prior to deployments. By measuring the rates of PRC losses from exposed SPMD, *in situ* k_e values can be derived for PRCs and compared with calibration k_e s of the native analogs. Any difference between field/exposure k_e s and laboratory calibration k_e s is due to differences in environmental conditions, after analytical variability is taken into account. When k_e calibration values and k_e exposure values deviate significantly, then adjustments of the laboratory calibration data can be made to better reflect actual sampling rates during an exposure. Thus, the PRC approach has similarities to the use of analytical internal standards for sample analysis, but serves an entirely different purpose. For additional details, see “PERMEABILITY/PERFORMANCE REFERENCE COMPOUNDS (PRCs)”, page 3-31.

DEPLOYMENT

To ensure successful use of the technology, investigators should be aware that once an SPMD is assembled, sampling of nonionic vapors is initiated. Thus, care must be used in handling, storage, and transport of SPMDs to and from exposure sites. Specific precautions used are given in “PRECAUTIONS/PROCEDURES DURING DEPLOYMENT AND RETRIEVAL”, page 4-5 of this guide. In general, practices aimed at minimizing potential procedural artifacts are based on logical precautions, which can be learned without special training and are related to good laboratory practices. A variety of deployment devices have been used in SPMD studies and at least one design is commercially available. Basically, common sense criteria apply to all acceptable designs that are needed to prevent contamination, damage/vandalism, and loss of the devices. For more details, see “STUDY CONSIDERATIONS”, page 4-1.

Section 3

THEORY AND MODELING

Huckins *et al.* (1993) described the basic theory related to the uptake and dissipation of contaminants by SPMDs and developed several mathematical models for estimating water concentrations from analyte concentrations in SPMDs. SPMD sampling rates were demonstrated to be independent of water concentration, while the amount of accumulated residues is proportional to dissolved environmental concentrations. This pioneering work focused on using residue concentrations in the SPMD lipid compartment to estimate ambient water levels of analytes. However, because of the convenience and enrichment of analytes achieved using the intact SPMD membrane for dialyses (Huckins *et al.*, 1993; Petty *et al.*, 1995; Rantalainen *et al.*, 1998) and the additional mass of analyte recovered from exposed membranes, most investigators analyze the whole SPMD. In this guide, we expand the lipid-based SPMD models to include the whole device.

The uptake of a chemical into the SPMD lipid compartment is given by (Huckins *et al.*, 1993)

$$C_L = C_w K_{LW} (1 - \exp [-k_o K_{mw} A t / K_{LW} V_L]) \quad (\text{Equation 3-1})$$

Where, C_L is analyte concentration in SPMD lipid, C_w is analyte concentration in water (note that relatively constant water concentrations are assumed), K_{LW} is the equilibrium lipid-water partition coefficient, k_o is the overall mass-transfer coefficient (expressed as velocity [e.g., cm / s], includes diffusion layer, biofilm or periphyton, and the membrane, as rate control is not assumed *a priori*), A is the surface area of the SPMD, K_{mw} and K_{mL} were defined earlier, t is time (when necessary, a diffusional lag or biofouling impedance term can be incorporated to adjust for non-zero intercepts), and V_L is the volume of lipid.

To minimize complexity (i.e., Occam's razor), the membrane can be expressed as a lipid-equivalent volume of sampling medium, and the equilibrium SPMD-water partition coefficient (K_{SPMD}) can be written as a one-compartment model as follows

$$K_{\text{SPMD}} = K_{\text{LW}} (V_{\text{L}} + [K_{\text{mw}} / K_{\text{LW}}] V_{\text{m}}) / V_{\text{SPMD}} = K_{\text{LW}} (V_{\text{L}} + K_{\text{mL}} V_{\text{m}}) / V_{\text{SPMD}} \quad (\text{Equation 3-2})$$

and

$$C_{\text{SPMD-E}} = C_{\text{w}} K_{\text{LW}} (V_{\text{L}} + K_{\text{mL}} V_{\text{m}}) / V_{\text{SPMD}} \quad (\text{Equation 3-3})$$

Where, V_{L} , V_{m} , and V_{SPMD} are the volumes of the lipid, membrane, and the lipid plus membrane, respectively, and $C_{\text{SPMD-E}}$ is the analyte concentration in the whole SPMD at equilibrium. Because we often use volume terms in this work instead of mass, it should be noted that both triolein and LDPE have about the same density, i.e., 0.91 (Roff *et al.*, 1971). The percent of $C_{\text{SPMD-E}}$ that an analyte has achieved is given by

$$E_{\%} = C_{\text{SPMD-t}} / C_{\text{SPMD-E}} \quad 100\% \quad (\text{Equation 3-4})$$

Where $E_{\%}$ is % of equilibrium and $C_{\text{SPMD-t}}$ is the concentration of the target compound at time t .

Knowledge of K_{SPMD} values is often needed to estimate water concentrations of chemicals of concern from SPMD concentrations. However, there are only a few published values of K_{SPMD} s (e.g., Huckins *et al.*, 1999). By using Equation 3-2 and regression models correlating widely available K_{ow} s to measured K_{LW} and K_{mw} values, K_{SPMD} s can be estimated. Using the data of Chiou (1985) and Petty *et al.* (1994), Hofmans (1998) correlated $\log K_{\text{ow}}$ values to $\log K_{\text{LW}}$ (triolein) and the best fit (S.D.= 0.18) is given by

$$\log K_{\text{LW}} = -0.1257 (\log K_{\text{ow}})^2 + 1.9405 (\log K_{\text{ow}}) - 1.46 \quad (\text{Equation 3-5})$$