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

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

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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 μm or 0.45 μm 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

EC_{50}	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 *in situ* sampling rates or to develop *in situ* 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
R^2	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
h_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 [µg/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 addition 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 [µg/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., ≈ 10 Å diameter) as the postulated 9.8 Å 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}s $\geq$ 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.

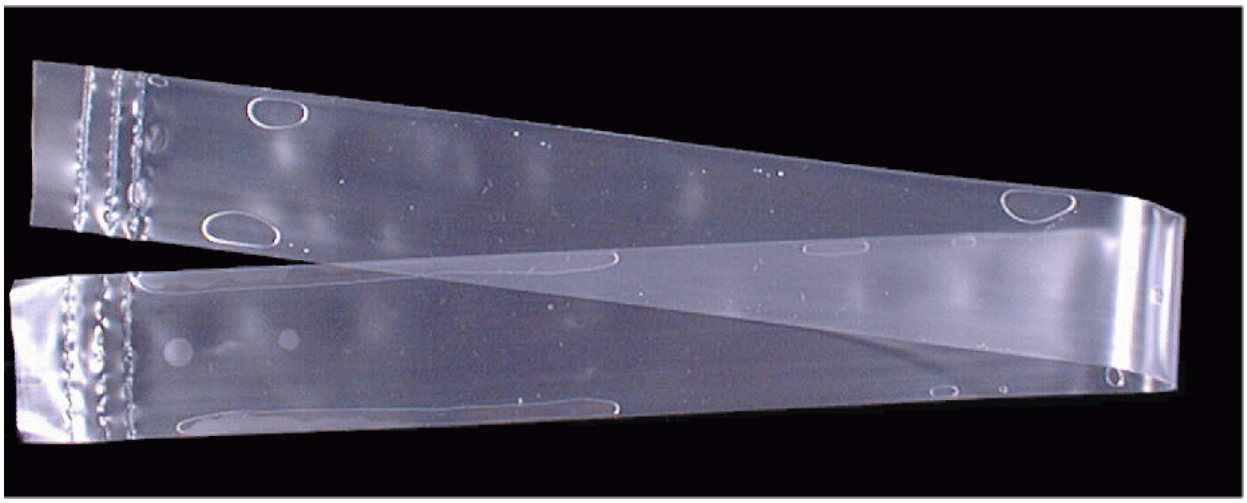

Figure 1-1. A standard lipid-containing SPMD with three molecular welds near each end. *Note the low interfacial tension causes intimate contact (i.e., the presence of a lipid film on the membrane interior surface) between the triolein and the membrane even where air bubbles exists.*

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 ^{o}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 by 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.

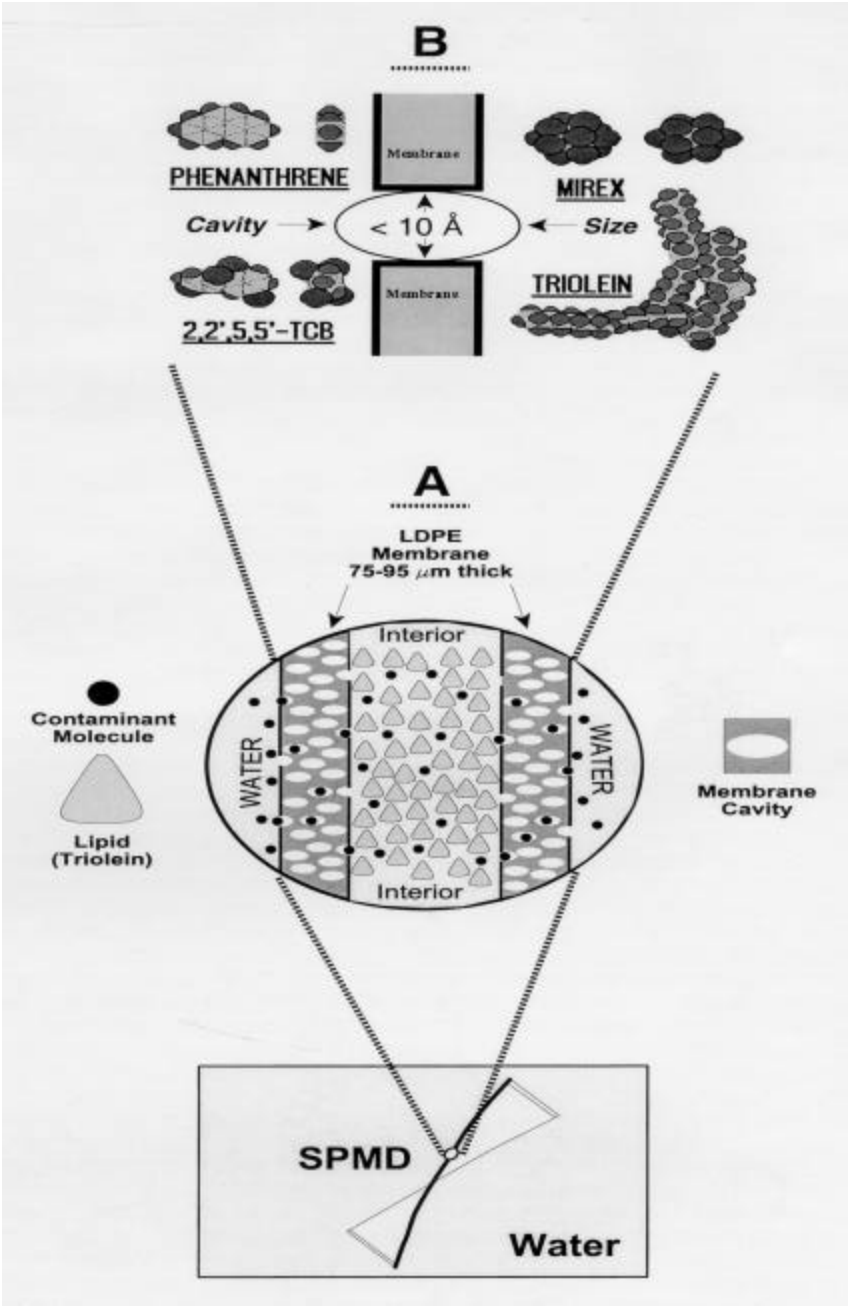

Figure 1-2. Exploded views showing the nonporous membrane size-exclusion phenomenon in the uptake and loss of organic compounds. ***Illustration "A" shows the movement of contaminant molecules through transient pores in the membrane and retention (membrane exclusion) of much larger lipid molecules. Illustration "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}s < 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}s > 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 “COMPARiSIONS 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}s ≥ 3.0) neutral organic compounds will be significantly concentrated above ambient levels. However, at log K_{ow}s < 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_as < 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 tropic 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}s $\leq$ 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)

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

and rearranging

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

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} s > 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.

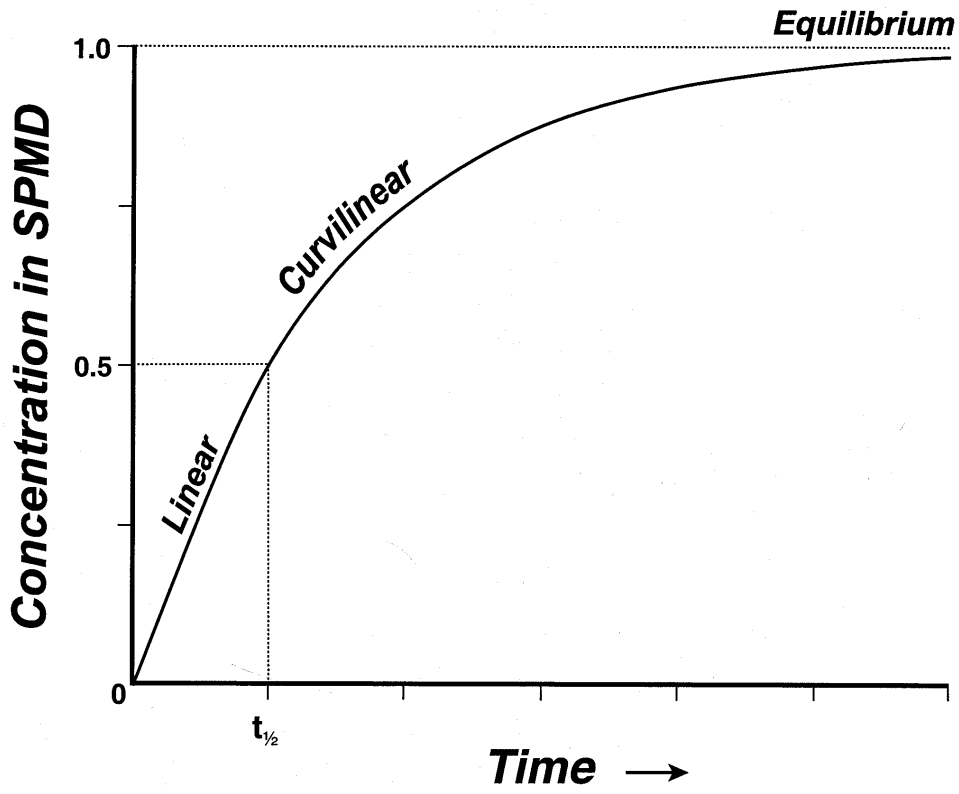

Figure 2-1. Plot of the Three Phases of SPMD Uptake. ***The amount of time that a chemical remains in the linear uptake phase or takes to reach equilibrium is dependant on molecular properties of the analyte, environmental conditions, and SPMD design. The time axis is given in half-times ($t_{1/2}$) 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 ($\leq$ 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 Å. 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, and1.9 ± 1.3 for pentachoroanisole. 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 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_ss) 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_es of the native analogs. Any difference between field/exposure k_es and laboratory calibration k_es 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)

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

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

$$K_{SPMD} = K_{Lw} (V_L + [K_{mw} / K_{Lw}] V_m) / V_{SPMD} = K_{Lw} (V_L + K_{mL} V_m) / V_{SPMD} \quad \text{(Equation 3-2)}$$

and

$$C_{SPMD\text{-}E} = C_w K_{Lw} (V_L + K_{mL} V_m) / V_{SPMD} \quad \text{(Equation 3-3)}$$

Where, V_L, V_m, and V_{SPMD} are the volumes of the lipid, membrane, and the lipid plus membrane, respectively, and $C_{SPMD\text{-}E}$ 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 $C_{SPMD\text{-}E}$ that an analyte has achieved is given by

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

Where $E_{\%}$ is % of equilibrium and $C_{SPMD\text{-}t}$ 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_{ow} values to log K_{Lw} (triolein) and the best fit (S.D.= 0.18) is given by

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

Note that PAHs with log K_{ow}s > 6.0 were not used in this analysis, because the model failed to adequately fit the data with their inclusion. Hofmans (1998) also correlated log K_{ow}s to log K_{mw}s of LDPE, using data from two authors (Lefkovitz *et al*., 1996; Reynolds *et al*., 1990). The best fit (S.D. = 0.23) is given by

$$\log K_{mw} = -0.0956 (\log K_{ow})^2 + 1.7643 (\log K_{ow}) - 1.98 \qquad \text{(Equation 3-6)}$$

Again, PAHs with log K_{ow}s > 6.0 were excluded.

Using the lipid-equivalent approach described earlier, and making no assumptions about the rate-limiting step in the SPMD uptake process, Equation 3-1 can be changed as follows to reflect the analyte concentration in the whole SPMD.

$$C_{SPMD} = C_w K_{SPMD} (1-\exp [-k_o K_{mw} A t / K_{Lw} (V_L + K_{mL} V_m)]) \qquad \text{(Equation 3-7)}$$

Also, in cases where aqueous boundary layer control of uptake rates is clearly dominant

$$C_{SPMD} \approx C_w K_{SPMD} (1-\exp [-k_w A t / K_{Lw} (V_L + K_{mL} V_m)]) \qquad \text{(Equation 3-8)}$$

Where k_w is the mass transfer coefficient in the aqueous boundary layer. When membrane control of uptake is clearly evident the following applies

$$C_{SPMD} \approx C_w K_{SPMD} (1-\exp [-k_m K_{mw} A t / K_{Lw} (V_L + K_{mL} V_m)]) \qquad \text{(Equation 3-9)}$$

MODELS FOR ESTIMATION OF WATER CONCENTRATION

Simplifying Equations 3-7, 3-8, or 3-9, and solving for ambient water concentration

$$C_w = C_{SPMD} / K_{SPMD} (1-\exp [-k_e t]) \qquad \text{(Equation 3-10)}$$

Where k_e is the group $k_w A / K_{Lw} (V_L + K_{mL} V_m)$ or $k_m K_{mw} A / K_{Lw} (V_L + K_{mL} V_m)$, which is an isotropic (exhibits the same kinetics for residue uptake and loss) exchange coefficient or rate constant (t^{-1}) for both overall uptake and elimination. Although this equation can be applied to all phases of SPMD uptake (Figure 2-1, page 2-6), it is most commonly used to estimate C_ws when analyte concentrations reach the curvilinear phase (i.e., $K_{SPMD} > C_{SPMD} / C_w > 0.693 K_{SPMD}$) of uptake during an exposure.

When $k_e t$ is very small (<<1) or $C_{SPMD} / C_w << K_{SPMD}$, chemical uptake is linear or integrative (Figure 2-1, page 2-6). Thus, in the linear region Equations 3-7 through 3-10 can be reduced to

$$C_w = C_{SPMD} M_{SPMD} / R_s t = C_{SPMD} / k_u t \qquad \text{(Equation 3-11)}$$

Where R_s (defined earlier as k_u / M_{SPMD}) is the whole SPMD (includes contributions of both the membrane and lipid) sampling rate in L or mL / d, and k_u is the uptake rate constant given in L or mL/d·g. More specifically, R_s is the group $k_w A$ (boundary layer control) or $k_m K_{mw} A$ (membrane control). Unlike the unit-mass specific k_u, R_s is a non-specific linear clearance constant (volume / time), which can relate to any size of SPMD or sampler. In this work, R_s values or calibration data are based or indexed to a standard-SPMD design (see "SPECIFICATIONS OF THE STANDARD SPMD", page 4-3) that contains 1 g of triolein. Thus, if an investigator uses a smaller or larger SPMD, the amount of accumulated residues must be adjusted to reflect the 1-g triolein size. Alternatively, the analyte's R_s value used in Equation 3-11 must be adjusted to reflect the V_{SPMD} or M_{SPMD} used in the exposure study.

The rate of uptake of a chemical by an SPMD is dependent on k_o, the overall mass transfer coefficient (the sum of individual mass transfer coefficients, i.e., molecular diffusivity in each layer divided by the respective thickness of layers), K_{mw}, the membrane-water

partition coefficient (membrane control), and A, the sampler external surface area. The sampling rate is related to the uptake rate constant k_u (mL or L / d·g) by

$$R_s = k_u V_{SPMD} d_{SPMD} = k_u M_{SPMD} \quad \text{(Equation 3-12)}$$

Where, d_{SPMD} is SPMD density (g/mL), M_{SPMD} is the mass (g) of the SPMD used, and R_s is related to k_e by

$$R_s = k_e K_{SPMD} V_{SPMD} d_{SPMD} \quad \text{(Equation 3-13)}$$

If $C_{SPMD}/C_w \approx K_{SPMD,}$ or the exponential group in Equation 3-7 and 3-10 becomes negligible (plateau of Figure 2-1, page 2-6), C_w can be determined using an equilibrium approach.

$$C_w = C_{SPMD\text{-}E} / K_{SPMD} \quad \text{(Equation 3-14)}$$

When calibration data (i.e., $K_{SPMD,}$ R_s, k_u, and k_e values) are available and analytes remain in the linear uptake phase (e.g., benzo[a]pyrene) or reach > 90% of equilibrium (e.g., naphthalene) Equations 3-11 and 3-14, respectively, are recommended for estimates of C_w. Equations 3-7 or 3-10 are used for compounds that reach the curvilinear phase of uptake (e.g., fluorene). If multiple measurements ($n \geq 3$) of C_{SPMD}s were made during the course of the study, C_w can be estimated by fitting Equation 3-7 or 3-10 to the data. However, the number of estimated parameters (e.g., C_w, k_e, and K_{SPMD}) should be no more than half the number of measured C_{SPMD} values. See examples of the use of Equations 3-10, 3-11 and 3-14 for the derivation of ambient water concentration in "Use of a Excel® Calculator for Estimating Water Concentrations from SPMD Data" in Appendix D "WATER CONCENTRATION EXTRAPOLATION", page D-3.

Groundwater Concentration Estimation

Aside from deployment considerations, sampling water in subterranean strata with SPMDs is generally straightforward, as environmental conditions are usually more constant than surface water and biological growths on the SPMD membrane surface are minimal. However, permeability in fine-grained strata can be very low, which may result in the depletion of target solutes at the membrane surface, i.e., uptake rate is limited by groundwater flux. Darcy's Law (Gustavson and Harkin, 2000a) permits estimation of groundwater flux (F_w) in a porous medium.

$$F_w = P_{hc} A \Delta h / L_f \qquad \text{(Equation 3-15)}$$

Where F_w has units of cm^3, L, or m^3/s or d, P_{hc} is the hydraulic conductivity of water or the coefficient of permeability in cm or m/s, which is analogous to the mass-transfer coefficient. The term A has been defined, Δh is the difference in hydraulic head between water at the top and bottom of the formation or strata, and L_f is the length of the formation. Typically, P_{hc} is measured directly or can be estimated from tables (Thibodeaux, 1996) for various strata. Values of P_{hc} in consolidated and unconsolidated media can be as low as $1 \times 10^{-9 \text{ to } -13}$ m/s. When SPMDs are placed in wells in this type of strata, the exchange volume of well water during an exposure (i.e., $F_w t$, where t is exposure time) should be compared to the clearance volume (i.e., $R_s t$) for the analyte of interest. If $F_w t >> R_s t$, then Equation 3-11 is appropriate for solving for analyte C_w. In the case of $F_w t << R_s t$, and assuming that desorption from suspended particulates or dissolution of chemicals of concern from non-aqueous phase liquids (NAPL) is insignificant, the following relationship is appropriate.

$$C_w = C_{SPMD} M_{SPMD} / V_{gw} \qquad \text{(Equation 3-16)}$$

Where V_{gw} is the volume of the water in the well. Equations 3-15 and 3-16 can be used to bracket or define the range of possible concentrations of dissolved contaminants in well

water. If the use of Equation 3-16 gives unreasonably high water concentration estimates, it is likely that desorption of analyte residues from natural organic phases or NAPLs in well water mediate ambient concentrations.

Sediment Pore Water

At the time of this writing, accurate derivation of undisturbed sediment pore water concentrations from analyte levels in SPMDs (buried in sediment) has not been demonstrated. The reason for this shortcoming stems from the potential dependency of SPMD uptake rates on the desorptive release rates of hydrophobic chemicals from sediment particles. Desorption rates of chemicals from sediments are often variable and are related to sediment organic carbon quantity, quality, and particle size. Even when the concentration of an analyte in the organic carbon phase of different benthic sediments is the same, variable pore water concentrations can be expected.

In the case of fine-grained sediments, particle interstices or water filled pore diameters are very small limiting the distance of aqueous diffusional steps. Rantalainen *et al.* (2000), have suggested that, at the points of contact between the SPMD membrane and sediment particles, the aqueous film thickness is very small. In addition, aqueous boundary layer thickness, which will be shown to be inversely proportional to mass transfer rates of hydrophobic compounds (see “RATE CONTROL”, page 3-11), is smaller for fine sediment particles. These observations suggest that SPMD uptake from sediments with a significant portion of clay and/or silt sized particles (i.e., < 60 μm) may be controlled by resistance in the organic carbon phase associated with sediment particles. Huckins *et al.* (1996) and Rantalainen *et al.* (2000) have observed a burst effect (i.e., an initial rapid rise in SPMD concentration [< 1 week], followed by a less rapid linear uptake phase) in the uptake of hydrophobic chemicals by SPMDs buried in sediment. This “burst effect” in fine grained sediments likely represents a switch in rate control, from the membrane or water phases to the sediment organic carbon.

A comparison of the uptakes (i.e., concentration factors, CF, [C_{SPMD}/C_w]) of 2, 2', 5, 5'-tetrachlorobiphenyl by SPMDs in flowing water (aqueous boundary layer control) and relatively fine grained sediment with 1% organic carbon (Huckins *et al*., 1993; Huckins *et al*., 1996) showed that the CF achieved in sediment exposed SPMDs was ca. 50% less than the CF of SPMDs in water alone. The magnitude of CFs achieved at times less than equilibrium time is dependant on the resistance to mass transfer. Obviously resistance to solute mass transfer was greater in the sediment than water suggesting significant resistance to transfer in the sediment organic carbon phase.

Rantalainen *et al.* (2000) have also shown that in benthic sediment environments, R_s can be written as the volume of organic carbon cleared of target chemical per unit time. This approach is clearly justifiable when the mass of a chemical in pore water is relatively low compared to the mass of chemical in the sediment organic carbon. Then from Rantalainen *et al.* (2000) the rate of sediment organic carbon clearance by SPMDs can be written as

$$k_{uoc} = k_u / K_{oc} = R_{soc} / M_{SPMD} \qquad \text{(Equation 3-17)}$$

To use this model one must also assume that K_{oc} remains constant with changing concentrations of chemicals in sediment and pore water, which may not always be the case. By determining k_{uoc} values for different sediments, the relative bioavailability of sorbed contaminants can be compared. Ultimately, SPMD and benthic organism comparisons will enable the use of SPMDs as a tool to assess chemical exposure to benthos.

SAMPLING APPROACH AND KINETICS

Selection of the most appropriate approach to estimate analyte C_w from concentrations in exposed SPMDs is dependent on whether the overall uptake was linear, curvilinear

or equilibrium was attained (Figure 2-1, page 2-6). The times an analyte will remain in the linear and curvilinear uptake phases, and the time required to approach equilibrium can be estimated when K_{SPMD} and R_s or k_u values are known for a specific set of environmental conditions. Also, note that the K_{SPMD} can be derived from Equations 3-2, 3-5, and 3-6 if the analyte's log K_{ow} is < 6.0. As indicated earlier, environmental exposure conditions must approximate or be indexed (i.e., use of PRCs) to calibration conditions to use this approach. Assuming the above information needs/conditions are met, the following equations can be used to compute appropriate exposure times and/or to select the modeling approach used in the estimation of analyte C_w.

$$t_{50} = t_{1/2} = -\ln 0.5\ K_{SPMD}\ V_{SPMD} / R_s = -\ln 0.5\ K_{SPMD} / d_{SPMD}\ k_u \qquad \text{(Equation 3-18)}$$

$$t_{90} = -\ln 0.1\ K_{SPMD}\ V_{SPMD} / R_s = -\ln 0.1\ K_{SPMD} / d_{SPMD}\ k_u \qquad \text{(Equation 3-19)}$$

$$t_{1/2} \approx -\ln 0.5\ K_{ow}\ V_{SPMD} / R_s \approx -\ln 0.5\ K_{ow} / d_o\ k_u \qquad \text{(Equation 3-20)}$$

Where, the mathematically identical terms t_{50} and $t_{1/2}$ are the first-order halftime (uptake) and half-life (elimination), respectively, t_{90} is the time required to reach 90% of steady state concentration (uptake) or to deplete 90% of the residues in an SPMD (elimination) and d_o is the density of octanol. Remember that Equations 3-2, 3-5 and 3-6 can be used to compute K_{SPMD} from K_{ow}. The first-order uptake of a chemical is linear during one $t_{1/2}$, curvilinear during > one $t_{1/2}$ to < four $t_{1/2}$s, and > four $t_{1/2}$s are required to reach > 90% of the equilibrium concentration in an SPMD. Alternatively, Equation 3-19 can be used to directly predict time to 90% of the equilibrium concentration.

Clearly, the use of Equations 3-18, 3-19 and 3-20 must be tempered with the fact that our R_ss and k_us were measured under conditions of low flow (< 0.5 cm/sec), negligible biofouling, and a temperature range of 10 °C to 26 °C. Thus, use of this modeling approach may require adjustments (PRC based) in calibration data to more accurately reflect the environmental conditions of exposures. In general, $t_{1/2}$ values of analytes

accumulated in SPMDs will be larger under conditions of low flow, low temperature, and heavy biofouling, and smaller under conditions of high flow, high temperature, and no biofouling (see "SYNOPSIS OF PERFORMANCE RELATED VARIABLES", page 2-9). The impact of these environmental factors on model selection is best addressed by the use of PRCs.

Using Equation 3-18 and assuming a 30 day exposure with environmental conditions similar to those used for PP PAH calibrations studies (see 18 °C data in Table 3-2, page 3-26), we can determine the appropriate models for the estimation of PP PAH concentrations in water. Because naphthalene approaches equilibrium in < 10 days, Equation 3-14, page 3-5 is appropriate. However, the uptake of acenaphthylene and acenaphthene is best described by Equation 3-10. Finally, all the rest of the PP PAHs would be expected to remain in the linear uptake phase and thus Equation 3-11 is appropriate.

The relative CF (C_{SPMD}/C_w) of residues in SPMDs also can be estimated through time by

$$CF = K_{SPMD} (1–\exp [–R_s t / V_{SPMD} d_{SPMD}]) \qquad \text{(Equation 3-21)}$$

Equation 3-21 allows an investigator to compare estimated SPMD CFs to reported BCFs (see "DATA FORMAT AND COMPARABILITY", page 5-14, for precautions related to this application). By multiplying the dimensionless CF times V_{SPMD}, the volume of water cleared of chemical by an SPMD can be determined, which is often needed for designing laboratory SPMD exposure studies.

Using Equations 3-2, 3-5, and 3-6 to derive K_{SPMD}, the total volume of water extracted by one mL of SPMD (i.e., $K_{SPMD} \cdot V_{SPMD}$ [membrane plus lipid]) at equilibrium can be estimated from an analyte's K_{ow}. This is possible because the K_{SPMD} can be viewed as the maximum volume of water cleared of chemical by a unit volume of SPMD. For example, if the derived log K_{SPMD} of a chemical is 4.0, then at equilibrium 10^4 mL of water would be

extracted by one mL of SPMD. This exercise is useful when assessing the utility of the SPMD approach for a group of chemicals with known K_{ow}s. Note that increasing the volume of a standard SPMD (see definition of a standard SPMD in "SPECIFICATIONS OF THE STANDARD SPMD", page 4-3) proportionally increases the membrane surface area and the total volume of water extracted at equilibrium.

Also, for a specific water concentration, the capacity or the mass of an analyte (M_a) in an SPMD at equilibrium is given by

$$M_a = K_{SPMD}\, V_{SPMD}\, C_w \qquad \text{(Equation 3-22)}$$

RATE CONTROL

The comparability of our experimentally derived SPMD uptake rates or exchange coefficients (calibration data) to actual values during environmental sampling generally depends on the similarity of laboratory and site exposure conditions. The choice of deployment devices for SPMD sampling can also play a significant role in laboratory and field comparability, because design features of the device may affect flow-turbulence at the membrane surface. When SPMD calibration and field conditions are dissimilar, the magnitude of the differences in lab and field uptake rates for an analyte depends in part on the source of analyte rate control. Thus, examination of potential rate-limiting barriers to analyte uptake by SPMDs is important. These barriers include the aqueous boundary or diffusion layer, any periphytic growths and particulates on the membrane surface (i.e., a biofilm with debris), and the SPMD LDPE membrane (Figure 3-1, page 3-13). The resistance or impedance (I) of each barrier to the mass transfer uptake and elimination) of analytes is generally assumed to be additive and independent. The overall resistance (I_o or $1/k_o$), to the uptake of a chemical is given by

$$I_o = 1 / k_o = I \;/ D_w + I \;/ D_b\, K_{bw} + I \;/ D_m\, K_{mw} = 1 / k_w + 1 / k_b\, K_{bw} + 1 / k_m\, K_{mw}$$

(Equation 3-23),

where all resistances to mass transfer have units of time per unit distance (e.g., s/cm), l is layer or barrier thickness, D is diffusivity (e.g., cm^2/s) in a particular region (note that the group D_m K_{mw} is the commonly used permeability coefficient [P] with the same units as D_m), and the subscripts w, b, and m, represent the aqueous diffusion layer, the biofilm layer, and the membrane, respectively.

Equation 3-23 shows that as the thickness of the biofilm and the aqueous boundary layers vary with exposure conditions (e.g., increased biofouling and/or turbulence), the impedance to chemical uptake will change and the rate-limiting step may switch to a different barrier. Also, if resistance changes in the rate-limiting barrier (e.g., increased turbulence in the sampling environment reduces aqueous boundary layer thickness), then chemical flux will either increase or decrease depending on the direction of the change. Remember, that any step or layer (in our case) with more than 50% of the total resistance is considered rate limiting. Examination of Equation 3-23 also indicates that in the absence of size-related diffusivity limitations that dramatically decrease D_m values, high partition coefficients for the membrane (K_{mw}) and biofilm (K_{bw}) phases effectively reduce the resistance to mass transfer in those layers. Under relatively low flow conditions (i.e., linear flow velocity < 1 cm/s) and using K_{ow} as an inverse measure of membrane resistance, research at the Netherlands Institute of Sea Research and CERC (Booij *et al*., 1997 and 1998; Huckins *et al*., 1997b) suggests that uptake rates of analytes with log K_{ow}s < 4.5 are largely controlled by the SPMD membrane (Huckins *et al.,* 1997b) and analytes with log K_{ow}s ≥ 4.5 are largely under aqueous diffusion layer control (Huckins *et al.,* 1997b; Booij *et al*., 1997; Booij *et al.,* 1998). Note that the actual point (i.e., analyte log K_{ow} value) that a switch from membrane to diffusion layer control occurs will vary. This variability is due to differences in flow regimes at different sampling sites, which affect boundary layer thickness and thus resistance to mass transfer. For some large molecules (i.e., cross sectional diameters > 10 Å) with very high K_{ow}s (i.e., log K_{ow} ≥ 7.0), membrane control may be reestablished. Also, as suggested earlier, biofilm control may occur for high K_{ow} compounds (log K_{ow} ≥ 6.0) when SPMDs are heavily fouled (see “Environmental Properties”, page 3-16).

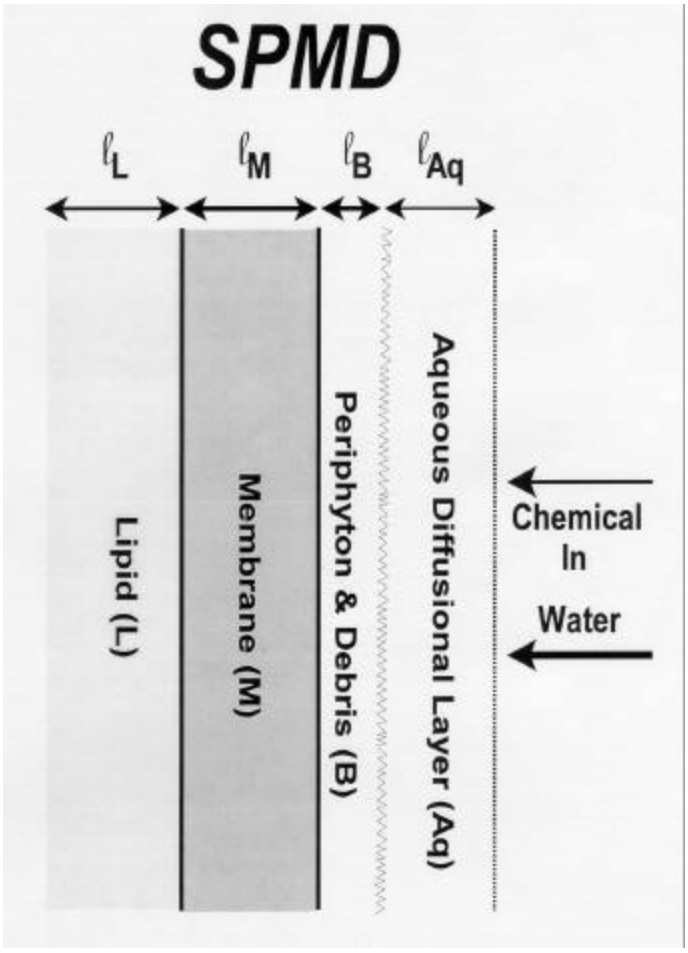

Figure 3-1. Profile of an SPMD showing potential barriers to analyte mass transfer or uptake. ***Note that the periphyton and debris layer may not always be present and*** ℓ ***is the hypothetical thickness of each respective barrier.***

FACTORS AFFECTING UPTAKE RATES

Several factors have been found to affect a chemical's sampling rate, which can be divided into three general categories: sampler design, molecular properties of analytes and environmental conditions. Note that for interrelated molecular properties, the division is largely operational.

Sampler Design

Although we recommend the use of the standard SPMD configuration, as described in the subsequent "STUDY CONSIDERATIONS", page 4-1, some discussion is warranted on how physical dimensions and membrane quality affect sampling rates. Because rate constants such as k_u and k_e are based on a unit mass of sampler, increasing the mass of an SPMD does not increase the magnitude of the uptake rates, unless there is an even greater increase in membrane surface area. In the case of R_s values, any increase in SPMD surface area with or without an increase in sampler mass will elevate sampling rates, as this constant is not indexed to a specific mass. Clearly, the area of the exchanging surface (A) is a key design feature for maximizing uptake and elimination rates of all analytes. The type (quality) and thickness of the nonporous membrane used in an SPMD (see criteria used for polymer selection in the "INTRODUCTION", page 1-2), only affects the uptake rates of analytes that remain under membrane control. Note that the maximum uptake rate for an SPMD is always achieved under boundary layer control (i.e., in a series of linked or interdependent steps, the rate of the first step sets the maximum limit of the rates of subsequent steps).

Molecular Properties

A number of structural features of contaminant molecules (i.e., presence of polar functional groups, electron density, molecular size and weight, and steric factors such as conformational freedom) can affect uptake/elimination rates by mediating compound solubility or chemical potential and diffusivity in the water, membrane, and lipid phases. Low solubility or high chemical potential in water and high solubility or low chemical potential in the membrane and lipid result in large K_{mw}, K_{Lw}, and K_{ow} values. The differential in an analyte's chemical potential in the water, membrane, and lipid phase is the driving force for uptake, after chemical concentration in the SPMD is higher than in the ambient water. In other words, a gradient in chemical potential (high to low) is the driving force for analyte mass transfer, not a concentration gradient.

In a membrane-controlled scenario, increasing K_{mw} and related membrane solubility generally result in a proportional decrease in resistance and an increase in R_s or k_u values. This phenomenon holds until resistance to mass transfer in the membrane falls below that of the aqueous boundary layer, causing the rate-limiting step to switch to the boundary layer. When rate control switches from the membrane to the diffusion layer, the importance of K_{mw} becomes negligible as shown by its absence in Equation 3-8, and mass transfer rate is mediated by the magnitude of k_w. Note that k_w is only affected by the effective boundary layer thickness and viscosity, and the molecular size-weight and/or molar-molal volume of analytes. When rising molecular weights and associated K_{ow}s of a group of homologues (e.g., PP PAHs) cause a switch to boundary layer control, R_ss and k_us will plateau and should slowly decline; based on the decrease in homologue molecular diffusivity and turbulent mass transfer rates. Assuming constant temperature, the general relationship (derived from Graham's Law with a correction for liquid diffusivity) for the effect of changes in molecular weight on diffusion coefficients or mass transfer rates under aqueous boundary layer control is given by

$$R_{sa} = R_{sb} (MW_b / MW_a)^{0.6} = (D_w A / l)_a = (D_w A / l)_b (MW_b / MW_a)^{0.6}$$

(Equation 3-24)

Where MW is molecular weight and the subscripts "a" and "b" represent compounds with different MWs. This equation combines several boundary layer theories. Because the exponent of Equation 3-24 is < 1.0, only relatively small changes in R_s values (≤ 1.5 fold) would be expected over a 200 Dalton range in analyte MWs. However, the data of Petty *et al.* (1994) and Meadows *et al.* (1998) show a steeper decline in R_s values for high K_{ow} compounds than predicted by Equation 3-24 (see Figure 7-1, page 7-3). Huckins *et al.* (1998a) pointed out that a similar steep decline in uptake rate has been observed for the gill (finfish) uptake of very hydrophobic contaminants (log $K_{ow} \geq 6.0$) with a relatively small range of molecular weights (see Figure 7-1, page 7-3). Several possible reasons for this phenomenon have been suggested (Huckins *et al*., 1998a), which include: 1) a second-order rise in effective resistance to mass transfer across the aqueous boundary layer as

cavity formation becomes increasingly difficult for large, very hydrophobic analytes, 2) a sharp reduction in residue solubility in the SPMD membrane and a concomitant increase in resistance to diffusant transfer in the LDPE (causes switch back to membrane control), as analyte molecules approach the maximal size of the transient cavities in the SPMD membrane (affects both R_s and K_{mw}), and 3) the partitioning of very hydrophobic solutes into POC and DOC, coupled with the lack of analytical methods that differentiate between dissolved and sorbed phases. In regard to the first reason for the apparent deviation from theory, Huckins *et al.* (1998a) have suggested that for very hydrophobic molecules, the potential exists for the formation of molecular dimers, which would dramatically decrease chemical flux across the aqueous boundary layer. Also, the potential role of the membrane in the phenomenon described above is not clear, because complex interactions of nonporous polymers with some diffusants have prevented the establishment of a precise relationship between molecular weight-size and polymer diffusion coefficients. However, for compounds with log K_{ow}s < 6.0, Hofmans (1998) developed the following empirical relationship (R^2 = 0.71, S.D.= 0.44) from data in the literature to correlate analyte molecular weight to LDPE membrane diffusion coefficients (D_m).

$$\text{Log } D_m = -2.3346 (\log MW) - 7.4707 \qquad \text{(Equation 3-25)}$$

Environmental Properties

These factors include water velocity/turbulence, biofouling impedance, and temperature. As suggested earlier, increasing current velocity/turbulence reduces the effective thickness of the aqueous diffusional layer, and thinner layers have less resistance to the mass transfer of chemicals. This means that, for compounds under aqueous diffusion layer control, sampling rates will be lower in quiescent waters than in more turbulent environments. In most environments the aqueous diffusion layer is not a simple stagnant film (Nernst layer) but rather a thin hydrodynamically complex region with an effective

resistance to mass transfer, often resulting in a steep concentration gradient of chemicals. Note that Hofmans (1998) has discussed in general terms the use of Schmidt numbers (i.e., the kinematic viscosity of water divided by the aqueous diffusion coefficient of the analyte), frictional velocities, and D_ws to estimate the effective thickness of SPMD aqueous boundary layers, but the detailed treatment is beyond the scope of this document. Also, this information is unnecessary if PRCs are used (see "PERMEABILITY/PERFORMANCE REFERENCE COMPOUNDS [PRCs]", page 3-31).

In the literature only limited data are available on the effective thickness (l) of aqueous boundary layers in different environmental systems, because l is sensitive to microscopic conditions that are hard to measure. However, various estimates suggest a range of ≈ 10-100 μm for high gradient streams and the surface waters of large lakes and seas (i.e., highly turbulent, with velocities of up to about 3 m/s), a range of ≈ 100μm to 500 μm for low flow environment such as small, low gradient streams and backwater areas, and ≈ 1000 μm for the quiescent sediment-water interface of deep stratified lakes or the deep-sea (Boudreau and Guinasso 1982). In the flow-through (< 1 cm/s) calibration exposures used to calculate R_s and k_u values given in this guide, we estimate the effective thickness of the aqueous boundary layer to range from ≈ 100 to 400 μm. Booij *et al.* (1998) observed about a four-fold increase in the SPMD sampling rates of PCBs and PAHs (wide range of K_{ow}s) by increasing the exchange velocity of experimental water thirty fold (i.e., 1 cm/s versus 30 cm/s). However, Huckins *et al.* (1997b) measured only about a 50% increase in SPMD sampling rates for flow velocities ranging from 0.004 cm/s to 0.2 cm/s (a 50 fold increase). Clearly, if SPMDs were placed unprotected in both stagnant and highly turbulent environments (assuming all other conditions are similar) differences in sampling rates for the same chemical could be large. In practice, the flow buffering effects of some deployment devices (e.g., EST's deployment apparatus shown in Figure 4-2, page 4-8) and less dramatic differences in flow/turbulence regimes at most study sites are expected to reduce variations in the flow velocity/turbulence at the membrane surfaces of environmentally exposed SPMDs.

Based on our experience, the thickness of a biofilm on exposed SPMD surfaces varies not only from exposure to exposure, but also over an individual membrane surface as well. However, biofilm regions of ≈ 1 mm thick have been observed on SPMD membranes in extended warm water exposures. Under a regime of constant low flow and temperature, Huckins *et al.* (1997b) reported that biofouling impedance or resistance to PP PAH uptake (i.e., a reduction in R_s or k_u values caused by periphytic growths and particulates on the SPMD membrane) increased with compound K_{ow} (Figure 3-2, page 3-19). The observed reduction in uptake rates ranged from about 35 to 70% for heavily fouled membranes (note that membrane surfaces were biofouled in a control pond before use in this exposure). Figure 3-2, page 3-19 appears biphasic in nature and the deviation of low K_{ow} acenaphthylene and acenaphthene (the two points nearest the x-y intercept) in the plot of PP PAH log K_{ow} versus % impedance (I_b) or fractional reduction in R_s values can be partly accounted for by the approach to equilibrium. The best fit ($R^2 = 0.83$) is given by

$$\%I_b = 100\% (1 - R_{s\text{-}b} / R_s) = 10 \log K_{ow} + 3.45 \qquad \text{(Equation 3-26)}$$

From Equation 3-26, the subscript "$_b$" refers to a biofilm or a biofouled SPMD. Clearly, the slope of this linear model is not steep. Thus, the impedance for analytes or PRCs with log K_{ow}s ≈ 4.5 is not very different than analytes with log K_{ow}s approaching 7.0.

Figure 3-2 suggests that biofilm uptake rate control is quite possible for high K_{ow} compounds during the latter part of some exposures. Fortunately, significant membrane biofouling is often delayed for about 7 to 14 days, because the attachment of periphytic organisms to the membrane is retarded (Hofelt and Shea, 1997; Gale, 1998) by the outward diffusion of triolein impurities (e.g., oleic acid and methyl oleate). In summary, increases in the coverage and thickness of periphyton on the SPMD membrane does

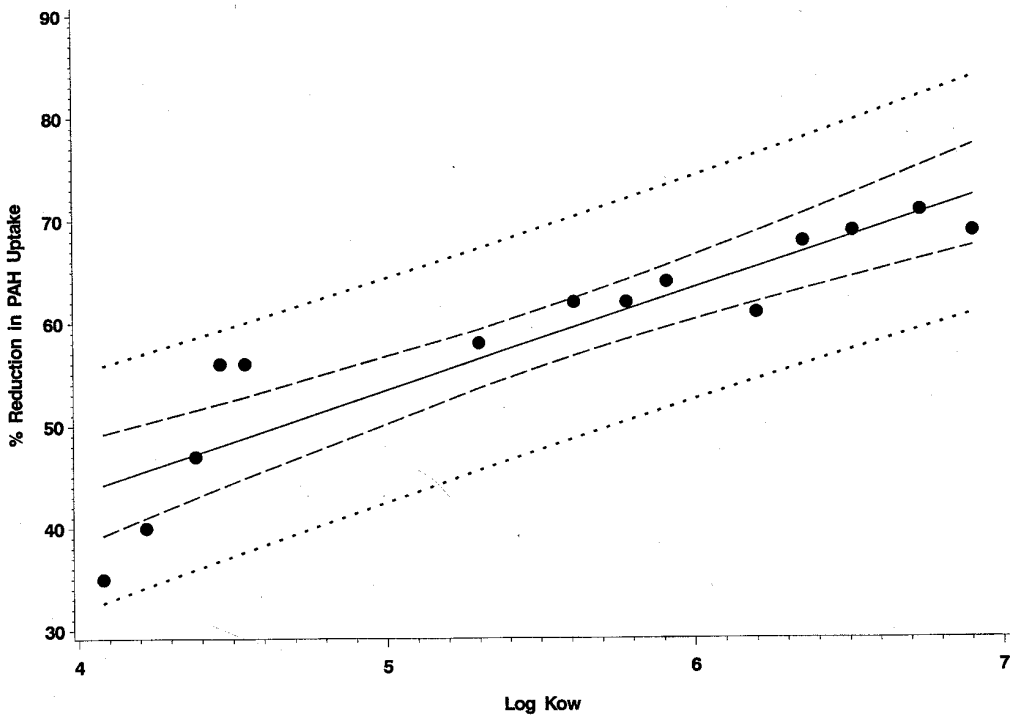

Figure 3-2. Linear-regression analysis of the biofouling mediated percent reduction (%I_b) in 21 day Native PAH Uptake, as a function of log K_{ow}. ***The dotted lines are the confidence band for individual predictions and the dashed lines are the confidence band for mean values. The correlation coefficient (R) is 0.91 (n=14).***

result in increases in the resistance to mass transfer of very hydrophobic compounds, which in turn reduces uptake rates.

Based on widely applied relationships such as the Wilke-Chang equation (Tucker and Nelken, 1982) and the Hayduk and Laude equation (Tucker and Nelken, 1982), analyte diffusion coefficients across the aqueous boundary layer are expected to be directly proportionally to temperature. Again, mass transfer through the aqueous boundary layer is more complex than simple molecular diffusion, but for a first approximation this modeling

approach is reasonable. The Hayduk and Laude model provides estimates of temperature mediated changes in aqueous diffusivity with an absolute error of < 6% and is given by

$$D_w = 1.326 \times 10^{-4} / \eta_w \, 1.14 \, V_D^{0.589} \qquad \text{(Equation 3-27)}$$

and D_w is related to R_s by

$$D_w = R_s l / A \qquad \text{(Equation 3-28)}$$

Where η_w is the viscosity (inversely related to temperature) of water for a specific temperature, and V_D is the LeBas molar volume (Tucker and Nelken, 1982). Using the above modeling approach for PP PAH calibration data, Huckins *et al.* (1999) predicted a 1.6 fold increase in D_w and R_s with a 16 °C temperature rise (i.e., 10 to 26 °C). The actual measured increase for PP PAHs with log K_{ow}s ranging from 5.20 to 6.35 was 1.7. However, outside this approximate order-of-magnitude range in K_{ow}s, measured increases in R_ss deviated significantly from predicted values. The phenomenon of reduced or nearly constant solute permeability with increasing temperature has been observed in nonporous polymers such as LDPE (Hwang and Kammermeyer, 1984) and (Comyn, 1985). Typically, increased temperature should enhance mass transfer in all media and the uptake of target analytes should exhibit Arrhenius dependences (i.e., plots of ln k_o A or ln k_o K_{mw} A versus 1/temperature in Kelvin should be linear). However, in membrane or biofilm controlled systems, non-ideal solute -polymer or -periphyton interactions may affect the activation energy required for molecular diffusion, increasing the complexity of the temperature-R_s relationship. Also, the K_{mw} or the K_{bw} may decline enough with increasing temperature to offset increases in D_m and D_b, respectively. Thus, it appears that the effects of temperature increases or decreases are less predictable for analytes under membrane and biofilm control.

In light of the aforementioned complications, Huckins *et al.* (1999) developed a multiple linear regression model to correlate the effects of changes in K_{ow} and exposure temperature on PP PAH R_s values. This empirical model is given by

$$R_s = 9317.5\ T - 336.78\ T^2 + 34015\ K_{ow} - 3177.6\ K_{ow}^2 - 3657.7\ T \cdot K_{ow} + 343.02\ T \cdot K_{ow}^2 + 130.63\ T^2 \cdot K_{ow} - 12.084\ T^2 \cdot K_{ow}^2 - 84343 \qquad \text{(Equation 3-29)}$$

Where T is exposure temperature in °C. Figure 3-3, page 3-22 shows the surface response of the model fitted to 14d PP PAH exposure data (Huckins *et al*., 1999). In the case of PP PAHs the maximum increase in compound R_s was < 2 fold over a 16 °C temperature rise, whereas R_ss of organochlorine pesticides (CERC laboratory calibration data) increased almost as much as four-fold over the same temperature range.

The temperature range used in the derivation of Equation 3-29 is not adequate for some environmental exposures. This is especially true for monitoring cold waters as temperatures down to –2 °C are possible, and our lowest exposure temperature was only 10 °C. To our knowledge, only one low-temperature calibration study has been conducted. Petty and Orazio (1996) examined the effect of –2 °C water (simulation of the lowest environmental water [liquid] temperature) on the uptake of selected compounds by modified SPMDs. Because triolein becomes a solid at ≈ 0 °C, silicone fluid with dispersed activated PX-21 carbon (powdered) was substituted for triolein in these SPMDs. Research has shown that this carbon, dispersed in silicone fluid and triolein, acts as an infinite sink (Huckins *et al*., 1995a; Petty and Orazio, 1996) for many compounds (e.g., PAHs). Note that the high sorption coefficient on carbon offsets the relatively low silicone fluid-water partition coefficient of test compounds (compared to analyte K_{ow}). In the linear uptake phase the modified SPMD configuration is expected to mimic standard SPMD uptake rates. Using this approach and an exposure temperature of –2 °C, the sampling rates (i.e., R_s values) of naphthalene and phenanthrene were 0.56 L/d and 0.59 L/d, respectively (Petty and Orazio, 1996). The R_s values for standard SPMDs exposed

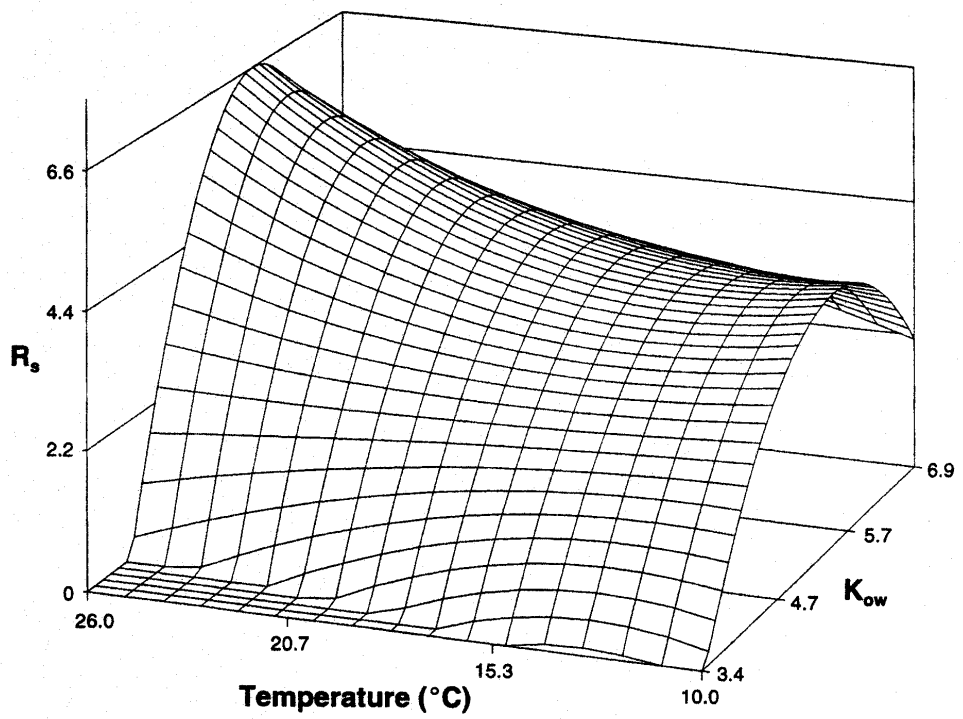

Figure 3-3. Effect of temperature and log K_{ow} on PP PAH R_s values (14 days, 100 ng/L) using a linear multiple regression model to show a surface response.

at 10 °C (Table 3-1, page 3-25) are 1.9 L/d (naphthalene) and 3.8 L/d (phenanthrene), respectively. Comparison of R_s values at the two temperatures shows that the sampling rates declined by about four fold for naphthalene and about six fold for phenanthrene. This steep decline in R_s values may relate to an exponential drop in LOPE chain segmental motility at low temperatures.

Most water quality parameters with the exception of temperature appear to have little effect on the magnitude of SPMD rate constants (i.e., analyte R_s, k_u, and k_e values), which are independent of analyte concentration, and relate to the volume of a medium cleared of chemical per unit time. However, factors such as salinity, POC, and DOC affect dissolved concentrations and thus do impact the amount of chemical accumulated by an SPMD.

For waters with high total organic carbon (TOC, i.e., DOC plus POC) levels, investigators may want to relate the dissolved concentration of an analyte, derived from SPMD levels, to total aqueous concentration (i.e., $C_{w\text{-}tot,}$ or mass of sorbed plus dissolved residues divided by the volume of the water). To that end, Meadows *et al.* (1998), developed the following model to correlate dissolved phase concentrations (estimated from SPMD concentrations) to the total concentration of PCBs in water

$$C_{w\text{-}tot} = (1 + TOC\ K_{oc} / M_w)\ C_{SPMD}\ V_{SPMD} / R_s\ t = (1 + TOC\ K_{oc} / M_w)\ C_w$$

(Equation 3-30)

Where K_{oc} is the organic carbon-water equilibrium partition coefficient and M_w is the mass of water.

CALIBRATION DATA

Estimation of the ambient water concentration of an analyte from its level in an exposed SPMD requires laboratory and/or *in situ* calibration data. These data consist of rate constants (i.e., R_ss, k_us and k_es) and partition coefficients (e.g., K_{mw} and K_{SPMD}), which are directly measured under controlled conditions or extrapolated from direct measurements. Several reports have been published with calibration data (Petty *et al.,* 1994; Huckins *et al.,* 1996; Booij *et al.,* 1998; Meadows *et al.,* 1998; Huckins *et al*., 1999). However, use of this data for estimating analyte water concentrations in different aquatic environments of concern is not always straightforward. This is because calibration conditions should approximate field conditions unless PRCs are used or *in situ* calibration is performed (Booij *et al*., 1998). Tables 3-1, 3-2, 3-3, 3-4, 3-5, and 3-6 on pages 3-25 to 3-30 summarize calibration data for PP PAH and organochlorine pesticides at three temperatures. The sampling rate data given in these tables were measured under low-flow conditions (< 1.0 cm/sec). Also, see “ADDITIONAL CALIBRATION DATA” in “Appendix

C", page C-1, for data on other classes of compounds. In the case of the studies leading to Tables 3-1 to 3-6 (pages 3-25 to 3-30), biofouling of SPMD membranes was negligible (SPMDs were treated weekly with a disinfectant). Therefore, the calibration data given in this section are directly applicable to sampling in environments with quiescent exposure conditions and minimal biofouling. For application of this data to SPMD deployments where exposure conditions are markedly different than calibration conditions see the following section on PRCs.

Table 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[a]

			10 °C Exposure Temperature			
Compounds	**log K_{ow}[b]**	**log K_{SPMD}**	**R_s (Ld^{-1})**	**CV (%)**	**K_u ($mL\ g^{-1}\ d^{-1}$)**	**K_e (d^{-1})**
naphthalene	3.45	3.36	1.9	1.5	350	0.150
acenaphthylene	4.08	3.63	2.3	8	430	0.100
acenaphthene	4.22	4.05	2.7	5	500	0.044
fluorene	4.38	4.21	3.0	5	560	0.034
phenanthrene	4.46	4.47	3.8	9	700	0.024
anthracene	4.54	4.67	2.9	9	530	0.011
fluoranthene	5.20	4.68	3.6[c,d]	---[d]	650	0.014
pyrene	5.30	4.79	4.5	15	830	0.013
benz[a]anthracene	5.91	5.32[e]	3.2	14	590	<0.003
chrysene	5.61	5.32[e]	3.7	18	670	<0.003
benzo[b]fluoranthene	5.78	5.55[e]	2.8	16	510	<0.001
benzo[k]fluoranthene	6.20	5.44[e]	2.9	18	530	<0.002
benzo[a]pyrene	6.35	5.11[e]	3.2	3	580	<0.004
dibenz[a,h]anthracene	6.75	4.83[e]	3.0	5	560	<0.008
indeno[1,2,3-cd]pyrene	6.51	4.51[e]	2.0	22	370	---[f]
benzo[g,h,i]perylene	6.90	4.04[e]	1.8	14	330	---[f]

[a] Values (0 of n = 9) given are from the 100 ng/L treatments studies and were calculated from days 4, 7, and 14 data (log K_{ow} > 4.4) and day 4 data (log K_{ow} < 4.4), and are recovery corrected.
[b] Preferred or selected values from Mackay *et al.* (1992a).
[c] n = 2, CV not determined.
[d] Recovery from SPMDs based on average of anthracene and pyrene values, because of interfering peaks (only in recovery studies).
[e] Equilibrium not reached.
[f] Not determined because K_{SPMD} not approached.

Table 3-2. Summary of SPMD PP PAH Sampling Rates, Exchange Coefficients, and Partition Coefficients Derived from an 18 °C Freshwater Flow-Through Exposure and Static Exposures[a]

			18 °C Exposure Temperature			
Compounds	**log K_{ow}[b]**	**log K_{SPMD}**	**R_s (Ld^{-1})**	**CV (%)**	**K_u ($mL\ g^{-1}\ d^{-1}$)**	**K_e (d^{-1})**
naphthalene	3.45	3.36	0.9[c]	41	170	0.074
acenaphthylene	4.08	3.63	1.4	3	260	0.060
acenaphthene	4.22	4.05	2.3	5	430	0.038
fluorene	4.38	4.21	1.7	6	310	0.019
phenanthrene	4.46	4.47	3.6	14	660	0.022
anthracene	4.54	4.67	3.6	17	660	0.014
fluoranthene	5.20	4.68	4.5[d,e]	---[e]	830	0.017
pyrene	5.30	4.79	5.2	10	950	0.015
benz[a]anthracene	5.91	5.32[f]	3.2	18	590	<0.003
chrysene	5.61	5.32[f]	4.8	11	890	<0.004
benzo[b]fluoranthene	5.78	5.55[f]	3.0	20	550	<0.002
benzo[k]fluoranthene	6.20	5.44[f]	3.9	13	710	<0.003
benzo[a]pyrene	6.35	5.11[f]	3.7	26	680	<0.005
dibenz[a,h]anthracene	6.75	4.83[f]	3.8	20	700	0.010
indeno[1,2,3-cd]pyrene	6.51	4.51[f]	3.0	17	360	---[g]
benzo[g,h,i]perylene	6.90	4.04[f]	1.9	31	340	---[g]

[a] Values (0 of n = 9) given are from the 100 ng/L treatments studies and were calculated from days 4, 7, and 14 data (log K_{ow} > 4.4) and day 4 data (log K_{ow} < 4.4), and are recovery corrected.
[b] Preferred or selected values from Mackay *et al.* (1992a).
[c] Beyond range of linear uptake.
[d] n = 2, CV not determined.
[e] Recovery from SPMDs based on average of anthracene and pyrene values, because of interfering peaks (only in recovery studies).
[f] Equilibrium not reached.
[g] Not determined because K_{SPMD} not approached.

Table 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[a]

			26 °C Exposure Temperature			
Compounds	**log K_{ow}[b]**	**log K_{SPMD}**	**R_s (Ld^{-1})**	**CV (%)**	**K_u ($mL\ g^{-1}\ d^{-1}$)**	**K_e (d^{-1})**
naphthalene	3.45	3.36	0.5[c]	44	90	0.039
acenaphthylene	4.08	3.63	1.7	4	310	0.072
acenaphthene	4.22	4.05	2.4	7	440	0.039
fluorene	4.38	4.21	2.8	1	520	0.032
phenanthrene	4.46	4.47	5.0	12	910	0.029
anthracene	4.54	4.67	4.6	31	840	0.015
fluoranthene	5.20	4.68	6.8[c]	14	1,260	0.028
pyrene	5.30	4.79	7.6	12	1,400	0.024
benz[a]anthracene	5.91	5.32[d]	4.7	17	860	<0.005
chrysene	5.61	5.32[d]	7.6	10	1,400	<0.006
benzo[b]fluoranthene	5.78	5.55[d]	3.3	33	610	<0.002
benzo[k]fluoranthene	6.20	5.44[d]	5.5	19	1,010	<0.004
benzo[a]pyrene	6.35	5.11[d]	5.4	10	980	<0.008
dibenz[a,h]anthracene	6.75	4.83[d]	4.7	8	860	<0.013
indeno[1,2,3-cd]pyrene	6.51	4.51[d]	3.4	17	630	---[e]
benzo[g,h,i]perylene	6.90	4.04[d]	2.4	9	440	---[e]

[a] Values (0 of n = 9) given are from the 100 ng/L treatments studies and were calculated from days 4, 7, and 14 data (log K_{ow} > 4.4) and day 4 data (log K_{ow} < 4.4), and are recovery corrected.
[b] Preferred or selected values from Mackay *et al.* (1992a).
[c] Recovery from SPMDs based on average of anthracene and pyrene values, because of interfering peaks (only in recovery studies).
[d] Equilibrium not reached.
[e] Not determined because K_{SPMD} not approached.

Table 3-4. Summary of SPMD Organochlorine Pesticides Sampling Rates Derived from a 10 °C Freshwater Flow-Through Exposure[a]

		10 °C		
Compounds	**log K_{ow}[b]**	**R_s (Ld^{-1})**	**C.V. (%)**	**k_u ($mL\ g^{-1}\ d^{-1}$)**
hexachlorobenzene	5.71	2.0	24	370
pentachloroanisole	5.48	2.9	23	530
α-BHC	3.86	0.9	45	170
lindane	3.71	0.7	63	130
β-BHC	3.86	---[c]	---[c]	---[c]
heptachlor	5.19	2.6	20	480
dacthal	4.26	0.6	44	110
oxychlordane	5.48	2.3	19	420
heptachlor epoxide	4.51	1.3	24	240
trans-chlordane	5.38	2.4	18	450
trans-nonachlor	6.35	2.7	18	500
cis-chlordane	5.38	2.6	17	480
o,p'-DDE	5.56	2.3	21	430
p,p'-DDE	6.14	2.8	29	510
dieldrin	4.60	1.3	17	240
o,p'-DDD	6.08	2.5	23	460
endrin	4.63	2.3	36	430
cis-nonachlor	6.20	2.2	16	400
o,p'-DDT	5.59	2.0	29	360
p,p'-DDD	5.75	2.3	17	430
p,p'-DDT	5.47	2.0	37	380
mirex	6.89	3.0	15	540
p,p'-methoxychlor	4.61	1.2	38	220

[a] Values ($\emptyset$ of $n \exists 3$) given are from the 100 ng/L treatments studies and were calculated from day 2 data for pesticides with log $K_{ow} < 4.4$ and the means of day 4, 8, and 16 data for pesticides with log $K_{ow} > 4.4$), and are adjusted for method recovery.

[b] Preferred or selected values from Mackay *et al.* (1997) and Syracuse Research Corporation On Line Log K_{ow} Estimator (KowWin), http:/esc.syrres.com/interkow/logkow.htm.

[c] Interference, samples not quantified.

Table 3-5. Summary of SPMD Organochlorine Pesticides Sampling Rates Derived from an 18 °C Freshwater Flow-Through Exposure[a]

		18 °C		
Compounds	**log K_{ow}[b]**	**R_s (Ld^{-1})**	**C.V. (%)**	**k_u ($mL\ g^{-1}\ d^{-1}$)**
hexachlorobenzene	5.71	2.6	24	480
pentachloroanisole	5.48	2.5	23	460
α-BHC	3.86	1.4	51	260
lindane	3.71	1.1	52	200
β-BHC	3.86	---[c]	---[c]	---[c]
heptachlor	5.19	---[c]	---[c]	---[c]
dacthal	4.26	1.8	52	320
oxychlordane	5.48	1.9	21	350
heptachlor epoxide	4.51	1.4	19	270
trans-chlordane	5.38	2.0	23	370
trans-nonachlor	6.35	1.9	20	360
cis-chlordane	5.38	1.7	17	300
o,p'-DDE	5.56	2.4	22	440
p,p'-DDE	6.14	2.7	27	500
dieldrin	4.60	2.6	20	490
o,p'-DDD	6.08	2.3	18	420
endrin	4.63	3.2	26	580
cis-nonachlor	6.20	2.0	22	370
o,p'-DDT	5.59	3.3	23	610
p,p'-DDD	5.75	2.5	23	460
p,p'-DDT	5.47	3.7	25	670
mirex	6.89	2.4	35	440
p,p'-methoxychlor	4.61	---[c]	---[c]	---[c]

[a] Values (0 of n = 3) given are from the 100 ng/L treatments studies and were calculated from day 2 data for pesticides with log K_{ow} < 4.4 and the means of day 4, 8, and 16 data for pesticides with log K_{ow} > 4.4), and are adjusted for method recovery.

[b] Preferred or selected values from Mackay *et al.* (1997) and Syracuse Research Corporation On Line Log K_{ow} Estimator (KowWin), http:/esc.syrres.com/interkow/logkow.htm.

[c] Interference, samples not quantified.

Table 3-6. Summary of SPMD Organochlorine Pesticides Sampling Rates Derived from a 26 °C Freshwater Flow-Through Exposure[a]

Compounds	log K_{ow}[b]	26 °C R_s (Ld^{-1})	C.V. (%)	k_u ($mL\ g^{-1}\ d^{-1}$)
hexachlorobenzene	5.71	5.6	19	1,030
pentachloroanisole	5.48	7.2	20	1,320
α-BHC	3.86	1.8[c]	23	330
lindane	3.71	2.3	43	420
β-BHC	3.86	1.6	51	290
heptachlor	5.19	6.8	12	1,240
dacthal	4.26	2.0	44	370
oxychlordane	5.48	5.6	16	1,020
heptachlor epoxide	4.51	5.3	8	980
trans-chlordane	5.38	6.0	8	1,100
trans-nonachlor	6.35	6.0	7	1,110
cis-chlordane	5.38	6.0	9	1,100
o,p'-DDE	5.56	6.0	21	1,110
p,p'-DDE	6.14	6.8	16	1,250
dieldrin	4.60	4.6	9	840
o,p'-DDD	6.08	5.5	20	1,010
endrin	4.63	7.6	35	1,400
cis-nonachlor	6.20	4.9	8	900
o,p'-DDT	5.59	4.0	17	740
p,p'-DDD	5.75	6.1	20	1,120
p,p'-DDT	5.47	4.1	21	760
mirex	6.89	5.8	12	1,070
p,p'-methoxychlor	4.61	2.5	26	460

[a] Values (0 of n = 3) given are from the 100 ng/L treatments studies and were calculated from day 2 data for pesticides with log K_{ow} < 4.4 and the means of day 4, 8, and 16 data for pesticides with log K_{ow} > 4.4), and are adjusted for method recovery.

[b] Preferred or selected values from Mackay *et al.* (1997) and Syracuse Research Corporation On Line Log K_{ow} Estimator (KowWin), http:/esc.syrres.com/interkow/logkow.htm.

[c] Potential interference in day 2 samples, used mean value of days 4, 8, and 16.

PERMEABILITY/PERFORMANCE REFERENCE COMPOUNDS (PRCS)

As previously stated, PRCs are (analytically) non-interfering compounds with moderate to relatively high SPMD fugacity, which are added to SPMD lipid prior to deployment. The use of PRCs can be viewed as an *in situ* calibration/recalibration approach. Figure 3-4 illustrates the principle of PRCs. The PRC approach is based on the assumption

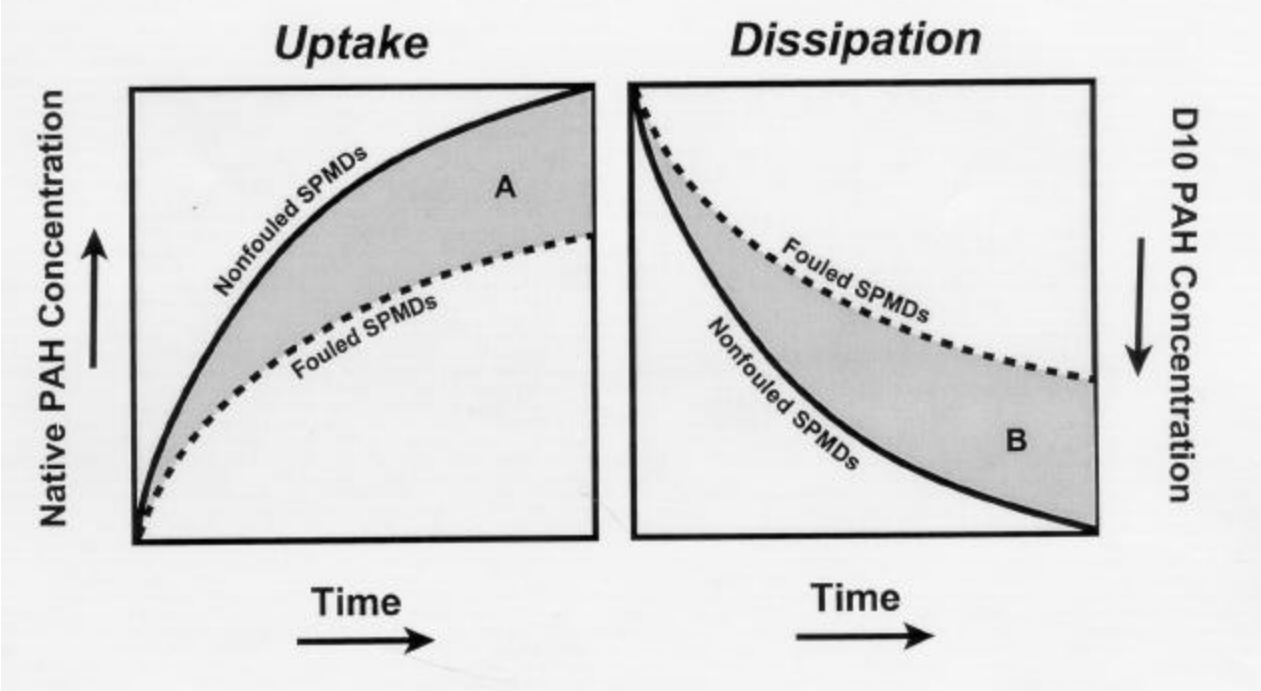

Figure 3-4. Illustration of why the permeability/performance reference compound (PRC) approach can be used to adjust SPMD sampling rates. *This example shows how a fouling induced reduction in the rate of a D_{10} PAH PRC (spiked into SPMD lipid) loss is proportional to the decrease in equivalent native PAH uptake rate. Note that area "A" = area "B".*

that *in situ* environmental uptake rates of analytes (i.e., $k_{u\text{-}f}$s or $R_{s\text{-}f}$s) can be derived from measurements of *in situ* loss rates of PRCs (i.e., $k_{e\text{-}PRC}$s) and from laboratory calibration data (i.e., K_{SPMD}s, $k_{e\text{-}cal}$, and $k_{u\text{-}cal}$s or $R_{s\text{-}cal}$s). This assumption is only true when uptake

and elimination rates are isotropic (i.e., the rate constants for overall uptake and elimination (k_e) are identical or when Equations 3-10 and 3-32 apply). Perdeuterated PAHs (note, this isotopic form is not normally found in the environment and can be separated from native forms) are often used as PRCs. However, investigators must ensure that the compounds used for PRCs differ from procedural [surrogate] and instrumental internal standards. Theory and experimental results (Huckins *et al*., 1994) suggest that the native and perdeuterated forms (e.g., PRCs) of the same compound have essentially the same k_es and k_us and that k_e values derived from uptake curves and elimination/clearance curves are essentially the same, when exposure conditions are identical.

Depending on the K_{ow}s of target analytes, candidate PRCs may have to include compounds that are representative of both membrane and diffusion layer control (see "Environmental Properties", page 3-16 for additional information). Also, general environmental conditions at sample sites and the duration of planned exposures are needed to help ensure that an acceptable range of PRC loss occurs during exposures. For example, PRC losses are enhanced under exposure conditions of warm turbulent waters. To prevent the complete loss of PRCs under this scenario, the use of compounds with moderately high log K_{ow}s (i.e., range of 4.5 to 5.3) may be necessary. Also, larger quantities of these PRCs and those with low log K_{ow}s (i.e., < 3.5), may have to be spiked into SPMDs. These precautions are necessary to ensure that changes in PRC residue concentrations can be statistically delineated from the C.V.s (%) for SPMD sample analyses (in general, the change in initial PRC concentrations [$C_{SPMD\text{-}0}$] should be ≥ 20% but ≤ 80%). Ideally, all PRC data should meet the criteria of $1-(C_{SPMD}/C_{SPMD\text{-}0}) \times 100\% > 3 \times$ C.V. (%) for PRC losses < 50% and $C_{SPMD}/C_{SPMD\text{-}0} \times 100\% > 3 \times$ C.V. (%) for PRC losses > 50%. Obviously, replication of PRC determinations is necessary to use the above approach. Even when PRC loss or retention is too great to use for the derivation of exposure adjustment factors (EAFs), information on the kinetic phase of analyte uptake (Figure 2-1, page 2-6) is still gained (Booij, 2000). For example, if a PRC with a log K_{ow} of

< 4.5 is completely lost during an exposure, then all analytes with log K_{ow} of < 4.5 will have attained equilibrium (i.e., K_{SPMD-E}). On the other hand, if no loss of a PRC with a log K_{ow} > 5.0 is observed then linear uptake can be assumed for all analytes with log K_{ow} > 5.0.

As suggested earlier, the rates of PRC losses (i.e., k_{e-PRC}s) from environmentally exposed SPMDs can be compared to the k_{e-cal}s derived for the same compounds during laboratory calibration studies (see k_es in Tables 3-1 to 3-6, pages 3-25 to 3-30) to determine the effects of exposure conditions on sampling. This approach permits the calculation of an exposure adjustment factor (EAF), which is defined by

$$EAF \equiv k_{e-PRC} / k_{e-cal} = (k_{u-f} / K_{SPMD-f}) / (k_{u-cal} / K_{SPMD-cal}) \quad \text{(Equation 3-31)}$$

and k_{u-f} can be derived from

$$k_{u-f} = K_{SPMD-f} (k_{e-PRC} / k_{e-cal}) (k_{u-cal} / K_{SPMD-cal}) \quad \text{(Equation 3-32)}$$

Note that field and lab K_{SPMD}s (i.e., K_{SPMD-f} and $K_{SPMD-cal}$, respectively) are not necessarily identical. The role of K_{SPMD} values in the EAF is discussed later (page 3-36) along with temperature effects. When temporal losses of PRCs are measured ($n \geq 3$), regression analysis can be used to determine PRC k_e values.

$$C_{SPMD} = C_{SPMD-0} \exp(-k_{e-PRC} t) \quad \text{(Equation 3-33)}$$

Where C_{SPMD-0} was defined earlier. Generally, a field blank containing a PRC is used to determine C_{SPMD-0}. If PRC levels in SPMDs are measured only at the beginning and the end of a field exposure, Equation 3-33 can be solved to permit a two-point derivation of k_{e-PRC}s (assuming first-order kinetics) as follows

$$k_{e-PRC} = \ln (C_{SPMD-0} / C_{SPMD}) / t \quad \text{(Equation 3-34)}$$

Obviously, these same equations (i.e., 3-33 and 3-34) can be used for determinations of $k_{e\text{-}cal}$s. If $k_{e\text{-}cal}$s of native PRC analogs were not measured in the laboratory they can be determined by rearranging Equation 3-13.

$$k_{e\text{-}cal} = R_{s\text{-}cal} / K_{SPMD}\, V_{SPMD}\, d_{SPMD} = k_{u\text{-}cal} / K_{SPMD} \qquad \text{(Equation 3-35)}$$

SPMD R_ss and k_us are affected by exposure temperature, biofouling, and aqueous boundary layer thickness, as shown in "FACTORS AFFECTING UPTAKE RATES", page 3-13. Use of EAFs to extrapolate $k_{u\text{-}f}$ or $R_{s\text{-}f}$ values of analytes other than the native equivalent of PRCs is dependent on the constancy of EAF ratios across a range of compound K_{ow}s and the availability of $k_{u\text{-}cal}$ values.

In controlled laboratory studies (Huckins *et al.,* 1994; Huckins *et al.,* 1997b), we demonstrated that the level of reduction (biofouling-induced) in the loss of perdeuterated phenanthrene (PRC) from SPMDs was similar to the level of reduction in the uptake rate of the native compound. However, Equation 3-26 shows that the ratio $R_{s\text{-}b}/R_s$ is somewhat dependant on the magnitude of the analyte's $K_{ow.}$ Fortunately, the slope of the regression line is not steep. The difference between the biofilm impedance (i.e., I_b, Equation 3-26) to the uptake of phenanthrene (log K_{ow} = 4.46) and I_b for benzo[*g,h,i*]perylene (log K_{ow} = 6.90) is only 13% which is often less than C.V.s for overall analytical procedures. In conclusion, it appears that the biofouling-mediated reduction in exchange rates of moderately hydrophobic PRCs can be used to estimate the biofouling impedance of more hydrophobic contaminants. Thus, I_b values determined for moderately hydrophobic PRCs appear to be applicable to compounds with log K_{ow}s within the range of 4.5 to 7.0.

Theory and the works of Booij *et al*. (1998) and Booij (2000) have indicated that the effect of changes in facial (SPMD membrane surface) velocity/turbulence is independent of analyte hydrophobicity or log K_{ow} (assuming boundary layer control, i.e., when analyte log K_{ow} > 4.5). Thus under an aqueous boundary layer control scenario, the EAF ratio for

deuterated (used as a PRC) and native (i.e., used in calibration) pyrene should be applicable to the derivation of not only pyrene $k_{u\text{-}f}$ and $R_{s\text{-}f}$ values, but to all other moderate to high K_{ow} PAHs as well.

The effects of differences in temperature on R_ss and k_us may have to be estimated (e.g., Equation 3-29 for PAHs) in part independent of PRC determinations (Booij, 2000). Equation 3-13 can be rewritten to investigate the potential for temperature effects on k_us and k_es.

$$k_u = k_w A / V_{SPMD} d_{SPMD} \qquad \text{(Equation 3-36)}$$

and

$$k_e = k_w A / K_{SPMD} V_{SPMD} d_{SPMD} = k_u / K_{SPMD} \qquad \text{(Equation 3-37)}$$

Obviously, written as above, these boundary layer controlled uptake and elimination models differ by the presence of K_{SPMD} in the denominator of the k_e equation. Theory suggests that K_{SPMD}s will vary with temperature and existing K_{SPMD} calibration data are mostly based on room temperatures (i.e., ≈ 24-30 °C). Initial results at our laboratory suggest an inverse relationship between temperature and $K_{SPMD.}$ Thus, unless K_{SPMD}s are measured or estimated for all study temperatures, PRC derived k_us from k_e/K_{SPMD} (i.e., PRC k_es and the use of Equations 3-2, 3-5, and 3-6) may differ from actual field k_u values, which are solely dependent on the magnitude of k_ws.

This discussion has centered on the use of PRCs in conjunction with laboratory calibration data. Booij *et al*. (1998) has pioneered the use of PRCs as a direct measure of the *in situ* uptake rates of analytes, with only minimal need for calibration data. The approach is based on the measurement of $k_{e\text{-}PRC}$s for PRCs (each site) with a wide range of K_{ow}s. Assuming that a sufficient number of $k_{e\text{-}PRC}$ values are measurable (i.e., data meets QC

criteria), regression analysis is used to extrapolate $k_{e\text{-}f}$s for target analytes. Regardless of the kinetic phase exhibited during an exposure, analyte K_{SPMD} must be measured or estimated. In the case of linear uptake throughout the exposure, Equation 3-32 is used to derive $k_{u\text{-}f}$s, which are used in Equation 3-11 to derive analyte water concentrations. When analyte uptake is non-linear, Equation 3-10 is used to estimate water concentrations.

In summary, the use of perdeuterated (all hydrogen atoms replaced with deuterium atoms) compounds appears to offer considerable promise as PRCs. They are commercially available, have physicochemical properties (excluding molecular weight) similar to their native analogues, are not found at significant levels in the aquatic environment, and generally can be separated from their native analogues by high-resolution gas chromatography. Based on our experience, the following perdeuterated PRCs are recommended: naphthalene, acenaphthene and fluorene for membrane controlled analytes and perdeuterated phenanthrene, anthracene and pyrene for diffusion layer controlled analytes (Caution: shading is advised to prevent PAH photolysis). Obviously, native compounds can be used for PRCs as long as they are not present in the environment sampled and are not otherwise used for analytical QC. As suggested earlier, class specific structural features are less important under diffusion layer control. Thus, PAH PRCs may be used for other classes of chemicals such as PCBs under these conditions. However, structural features of chemical classes are important under LDPE membrane control (Roff, 1975) and possibly under biofilm control. In conclusion, the PRC approach should improve the accuracy of water concentration estimates, when current velocity/turbulence and biofouling affect SPMD sampling rates. Until the accuracy of the PRC approach for thermal effects can be further evaluated by determing K_{SPMD} values at the range of potential environmental temperatures, external measurement of R_ss at multiple temperatures is advised.

Section 4

STUDY CONSIDERATIONS

SOURCE AND PREPARATION OF SPMDS

The SPMD technology is the subject of two Federal Government Patents (Nos. 5,098,573 (Huckins *et al.,* 1992) and 5,395,426 (Huckins *et al.,* 1995b) and a Canadian Patent (No. 2,037,320). A private company, Environmental Sampling Technologies (EST), a division of Custom Industrial Laboratories, 1717 Commercial Drive, St. Joseph, MO 64503, has been granted an exclusive license to the technology (including the organic solvent dialysis procedure for analyte recovery from whole SPMDs) by the US Department of Commerce. Currently, SPMDs are only available from EST or sub licensees such as ORIGO Hb, Trehörningen 34, 5-922 66 Taveljö, Sweden (Europe). However, as the inventors of SPMDs, CERC scientists continue to perform research on the development and refinement of the technology. Herein, most aspects of the preparation process, as performed at CERC and EST, are presented to illustrate selected quality control issues.

Preparation and Evaluation of Materials

Careful attention to precleaning all SPMD components is a critical part of achieving low levels of interferences in SPMD samplers. To that end, the layflat-LDPE tubing (an EST standard material, which contains no antioxidants, slip additives, plasticizers, etc.) is treated with high purity hexane (typically three, 24 hour treatments with a minimum of 2.2 mL of hexane per cm of tubing [2.5 cm wide]). This step is designed to remove most of the lower molecular weight polyethylene waxes (oligomers), which are present in all LDPE, and other potentially interfering (analytical) compounds present in or sorbed by the LDPE.

Following this treatment, representative samples of the cleaned up LDPE membrane are processed by dialytic extraction, enriched (treatment with size exclusion chromatography [SEC] to remove LDPE oligomers), and examined with gas chromatography using electron capture (GC-ECD). Also, samples are generally subjected to analysis using either a GC equipped with a flame ionization detector (FID) or GC-mass spectrometry (MS). If these analyses show no or only negligible levels of interferences, the cleaned tubing is stored until use in sealed metal cans under an inert atmosphere (usually argon).

The triolein (95% purity) is also examined for contaminant residues before use in SPMDs. The procedure includes dialysis of a 10 g portion of triolein, SEC to remove the co-dialyzed LDPE oligomers and lipid components, and analysis of the enriched extract by GC-ECD and GC-FID or GC-MS. After demonstrating that no interfering compounds are present at levels of concern, the lipid (sealed under an inert gas in glass ampoules) is stored at –15 ^{o}C until assembly of the SPMDs.

SPMD Assembly

All SPMDs are assembled in an environmentally controlled room, equipped with an activated carbon air filter for the removal of vapor phase contaminants. SPMDs can be prepared in almost any lengths after allowance of space for the molecular welds (i.e., ≈ 2.5 cm for each end). However, different lengths of SPMDs must maintain the standard SA-V ratio (i.e., membrane surface area in cm^2 divided by total volume of the SPMD, cm^3 or mL), which is 460 cm^2 / mL triolein (see “SPECIFICATIONS OF THE STANDARD SPMD”, page 4-3 for a detailed description of standard SPMDs). Because at room temperature, triolein is viscous and can cling to surfaces, a pipetter equipped with a total displacement plunger is employed to ensure accurate volumetric delivery. Afterwards, the triolein is formed into a thin film along the length of the membrane, the air is forced out, and the membrane is heat sealed at both ends, which normally consists of three to four seals at

each end. Tether loops of LDPE tubing (no triolein) can be welded to both ends of the SPMD membrane to facilitate deployment.

To enable universal comparability of SPMD data, a standard SPMD design must be defined and some of the standard devices must be included in studies using non-standard samplers. Because commercially available SPMDs are modeled after the original USGS design, are of uniform construction, are used globally, and represent the configuration used in most calibration studies, we recommend that specifications of these devices be considered as the standard.

SPECIFICATIONS OF THE STANDARD SPMD

The design of commercially available SPMDs consists of a specified length (typically, 91.4 cm [distance between the inter welds or the length of the triolein containing portion]) of 2.5 cm wide layflat LDPE tubing (additive free, wall thickness 70-95 μm), containing 95% purity triolein (1 mL used for the 91.4 cm length). After heat-sealing the triolein inside the LDPE tube, the resulting SA-V (membrane surface area to total SPMD volume) ratio is ≈ 90 cm^2/mL or ≈ 460 cm^2/mL of triolein, and the device consists of ≈ 20% triolein. For the 1 mL triolein configuration, the whole device typically weighs ≈ 4.4 to 4.6 g.

Thus, any length of SPMD with an SA-V ratio of ≈ 460 cm^2/mL of 95% triolein, a ≈ 0.2 lipid-to-membrane mass ratio, and a 70-95 μm wall thickness is considered a standard SPMD (Figure 1-1, page 1-3). However, the aforementioned QC requirements of the membrane and triolein must be met. As stated earlier, most SPMD calibration data and field data are based on the standard configuration; thus any fundamental change in design should be considered in light of the obvious advantages of data comparability.

STORAGE AND TRANSPORT

SPMDs that must be stored prior to field deployment should be maintained under clean argon at ca. –15 °C, in the gas-tight sealed metal cans provided by the supplier (Figure 4-1). The canned samplers should be shipped to the field in efficient coolers specifically designated for SPMD transport. While it is not always essential to transport the SPMDs to the field at low temperatures (the SPMDs are in an inert atmosphere until the seal on the can is broken), it is always good practice to maintain the samplers frozen or at near freezing temperatures. When PRCs are used, SPMDs should be maintained at freezing- or near-freezing conditions during transport to minimize losses of these QC compounds.

A variety of coolants can be used for shipping, which include ice, blue ice, and dry ice. Following retrieval from the exposure medium, the SPMDs should immediately be sealed inside the same metal cans and transported (frozen or near frozen) back to the analytical laboratory in the same cooler. If it is necessary to delay the shipping of exposed SPMDs more than a few hours, then they should be stored frozen at ≤ –15 °C in the sealed metal cans. Caution: failure to maintain exposed SPMDs under freezing conditions can result in significant losses of analytes with relatively high fugacities (e.g., naphthalene). However, no losses of 2,4,5-trichlorophenol (SPMD fugacity is relatively high) from SPMDs, stored at –15 °C for 6 months, were measurable (unpublished data, CERC, USGS).

Figure 4-1. For storage and shipping, SPMDs (shown on a deployment apparatus rack) are placed in a clean metal can, flushed with argon, and sealed with a gas tight lid.

PRECAUTIONS/PROCEDURES DURING DEPLOYMENT AND RETRIEVAL

Because SPMDs sequester a wide variety of solutes or vapors of hydrophobic chemicals, care must be used to prevent inadvertent contamination of the devices. Of particular concern is the fact that SPMDs concentrate vapor phase chemicals at about the same rate as aqueous solutes (note, on a volumetric basis clearance rates of air are much higher than water because of the density difference between air and water phases). For example, based on phenanthrene's aqueous R_s of 3.6 L/d for a standard 1-mL triolein SPMD @ 18 °C, we estimate a phenanthrene vapor phase R_s of ≈ 2 m^3/d. In other words, phenanthrene vapors in 1.4 L of air are sampled each minute when an SPMD is exposed to the atmosphere.

Overall, proper handling of SPMDs consists of logical precautions, which can be learned without special training and are related to good laboratory practices. SPMDs are used in a wide variety of aquatic ecosystems, ranging from wetlands, lakes, and highly energetic rivers such as the Missouri River, to estuaries and ocean environments. However, the following practices and considerations apply to all deployment scenarios. 1) Before aqueous deployment and prior to retrieval, carefully inspect study sites for sources of vapor-phase contaminants, which include fumes from engines, oils, tars, gasoline, diesel fuel, paints, solvents, cigarette smoke, asphalt pavement, etc, and record any findings for each site. Clearly, the importance of reducing atmospheric exposure time during deployment and retrieval is underscored when in the presence of vapor phase contaminants. 2) Keep in mind that at some sites, atmospheric levels of certain target compounds may be higher than concentrations in receiving waters and once the SPMD is exposed to site air, sampling begins. 3) Ensure that cans with SPMD field blanks (see "QUALITY CONTROL", page 5-1, for a definition of QC samples) are open to the air while sample SPMDs are being deployed and retrieved. The field blank SPMDs provide a record of any chemical accumulated in SPMDs during transport, deployment and retrieval. 4) If waterborne chemicals are visible as surface layers of oils, tars, gasoline, etc., or a biofilm is visible on the surface of the water, where target compounds are potentially elevated (i.e., relative to the water column), precautions may be needed to reduce contamination during aqueous insertion of deployment devices. 5) Hand lotions, perfumes, colognes, powdered gloves (use powder free gloves), etc, should not be used when handling samplers or deployment devices, as they likely contain chemicals accumulated by the SPMDs. 6) The procedure for retrieval of the SPMDs is essentially the reverse of the deployment sequence and the same precautions apply. Following retrieval, immediately place the SPMDs back into the same metal cans, as provided by the supplier, and seal the lid on the can (Caution: if the lid is not perfectly sealed on the can, contamination of SPMDs during transport back to the analytical laboratory is highly probable). Place the cans containing the SPMDs into a cooler and maintain frozen pending and during shipment to the processing laboratory.

In summary, key quality-control considerations during deployment and retrieval of SPMDs are given. It is particularly important to remember that sampling and exchange of PRCs begins as soon as SPMDs are exposed to air and that target chemicals may be at higher concentrations in the air at a site than the water column under study.

DEPLOYMENT METHOD

SPMDs have been successfully deployed in a variety of containment systems (i.e., deployment apparatus/device). The commercially available deployment apparatus shown in Figure 4-2, page 4-8 is the most widely used system. Regardless of the choice of SPMD deployment structure, certain generic guidelines should be used in its design and construction. These include, 1) metal containment structures must be free of cutting oils or other potential interferences, 2) use of plastic components should be minimized (Teflon is an exception), due to the possible presence of leachable organic residues, and in some cases, competitive sorption of analytes by the plastic, 3) the design of the structure should minimize abrasion of the LDPE membrane even in turbulent environments, reduce site-to-site differences in the effective thickness of the SPMD aqueous boundary layer (note that container designs that baffle water flow can be used to accomplish these goals), and maintain adequate exchange rates at the membrane surface, 4) once the SPMDs are mounted in the deployment device, the lipid containing portion of the layflat tubes must not make contact with container walls (excluding boundary layers, ambient water must have unrestricted access to membrane
surfaces), and when the tubing is looped or in a “zigzag” configuration, self-adherence of loops must be prevented (e.g., the use of a Möbius configuration by Lebo *et al*., 1992) if water turbidity is low and the deployment apparatus does not provide complete shading of mounted SPMDs, a shading structure may be required for analytes that undergo

Figure 4-2. A commercially available stainless steel deployment apparatus, which has a capacity of 5 Standard SPMDs. Each SPMD is placed on separate racks and the five racks are held in place by a threaded center pin as shown in the picture.

photolysis (e.g., certain PAHs), 6) the structure should be adequately tethered to prevent loss during minor flood events, and 7) since vandalism is always a potential problem in the field, the structure should be amenable to hiding. As a final note,
designs that minimize “silting in” should be used if deployments are at the sediment-water interface.

EXPOSURE DURATION AND ENVIRONMENTAL VARIABLES

The appropriate exposure duration is dependent on a number of factors. These include: 1) the types of target analytes and analytical sensitivity needed (i.e., MDLs and MQLs), 2) the choice of sampling approach (i.e., integrative versus equilibrium), 3) time resolution

desired for changes in analyte concentration, and 4) environmental or site variables, such as expected level of biofouling, temperature, turbulence-flow rate, and the probability of possible damage to or loss of devices due to turbulent-advective conditions/events (i.e., floods, storms, tidal fluctuations) and vandalism. Obviously, tradeoffs commonly occur when the decision tree involves these types of interrelated factors, which require prioritization of project goals.

Because certain environmental variables nearly always have some effect on the uptake of target analytes from water, regardless of analyte types or sampling approach (i.e., kinetic or equilibrium), a record should be kept on site conditions during exposures. Relevant data include temperature (a minimum of the beginning and end of the deployment), the visual extent of fouling (i.e., light, medium, heavy, none), and an estimation of turbulence-flow rates (i.e., cm/s). This type of data is helpful even when PRCs are used, because it provides information on the possible causes of significant differences between calibration R_ss and field derived R_ss.

As with any research or monitoring approach, notes describing the site, events occurring during deployment and retrieval, etc. should be recorded. In general, complete notes and as much information as possible should be collected. It will prove invaluable if QC-related questions arise during sample processing and analytical procedures, and will often be helpful in conducting site assessments.

Section 5

SPMD ANALYTICAL CHEMISTRY

Procedures used for the analysis of SPMDs samples are similar to those utilized for the determination of organic contaminant concentrations in the tissues of aquatic organisms. However, extracts from aquatic organisms can vary widely in lipid quantity and composition, while SPMD extracts (i.e., dialysates) are more uniform, well characterized, and generally contain less lipid. Thus, methods for the analysis of SPMD samples are generally more amenable to standardization than those used for aquatic organism tissues.

QUALITY CONTROL

The application of appropriate Quality Control (QC) procedures/parameters is a mandatory consideration in both SPMD deployment and sample analysis. QC samples should address issues of SPMD-materials background, and contamination incurred during transport, deployment and retrieval, and storage, as well as analyte recovery during sample processing, enrichment, and fractionation operations. The exact level of QC required should be determined during the development of a project's experimental design. In general, QC samples represent 20-50% of a "sample set". We operationally define a laboratory sample set as a group of samples (includes both exposed SPMDs and QC samples from the same study) that are processed and analyzed together. The number of samples in a sample set generally ranges from 2 to 24. The upper limit of a sample set size is often constrained by analytical procedures (e.g., column chromatography) where a particular step must be monitored. For projects needing stringent QC, control charts are recommended to monitor analyte recoveries throughout a project (see Taylor, 1987, for specifics). Briefly, during each quarter of a project, the last 20 observations of recoveries

from QC spikes are used to generate a control chart (note computer programs such as SigmaPlot® are used at CERC). Control limits are established for the analytical process as described by Taylor (1987). When control limits are exceeded, sample analyses are suspended until the problem step(s) can be identified and corrected.

Herein, we describe the basic QC samples/parameters related to the performance of SPMD studies, and elucidate their role in the conduct of studies. Also, an overview of SPMD analytical procedures, and data applicability is given.

SPMD-Fabrication Blanks

This type of QC blank consists of individual SPMDs, prepared as part of a batch or lot of SPMDs of the same size and material, which are assembled for a specific project. They are maintained frozen at –10 to –20 °C in the laboratory (sealed in metal cans under argon) until the analysis of exposed SPMDs. Processing and analysis of SPMD-fabrication blanks is concurrent with and identical to that of deployed SPMDs. The primary purpose of this type of QC sample is to account for any background contribution due to interferences from SPMD components, and for contamination incurred during laboratory storage, processing, and analytical procedures.

SPMD-Process Blanks

This type of QC blank consists of a group of SPMDs, made just prior to initiation of the analysis of an SPMD sample set. Operationally, the only difference between SPMD-process blanks and SPMD-fabrication blanks is the time of preparation and the lack of a storage period. Use of this type of blank is in part limited because only two US laboratories (EST and CERC) assemble SPMDs (see “SOURCE AND PREPARATION

OF SPMDS", page 4-1). If the numbers of SPMD-fabrication blanks are inadequate, laboratory-SPMD blanks are used to determine analyte recovery and the precision of the overall analytical method. Also, this type of QC sample can be used to determine any potential effects of storage or changes in batches or lots of SPMD materials.

Reagent Blank

These blanks consist of aliquots of all solvents (volumes identical to those used for SPMD samples) used during the processing, enrichment, and instrumental analysis of an SPMD sample, that are carried along with SPMD samples through the entire analytical procedure. This type of QC sample (at least one for each sample set) provides information on background due to laboratory reagents and procedures. The use of reagent blanks is strongly recommended, because they greatly facilitate diagnosis of any interference problems encountered during SPMD analysis.

Field-Blank SPMDs

These blanks consists of individual SPMDs (at least one per sampling site) obtained from the same manufactured lot or batch as the SPMDs used in an exposure study. Field-blank SPMDs are used as QC samples for transport, deployment and retrieval (note that these SPMDs are sealed back in the same shipping cans and stored frozen during the exposure period), and for spiking with PRCs. The field–blank SPMDs are treated the same as deployed devices, with the exception that they are not exposed to waters at study sites. These field blanks account for potential contamination during SPMD transport (both to and from study sites), and during exposure to site air while deploying and retrieving SPMDs. As discussed earlier, the purpose of PRC-spiked field-blanks is to assess the effects of environmental conditions on analyte sampling rates (i.e., derivation of an EAF) or for *in situ*

calibration (Booij *et al*., 1998). Generally, both types of field-blank SPMDs are critical to the success of environmental studies.

As suggested earlier, field-blank SPMDs are taken to the field in sealed metal cans and one or more cans are opened to the atmosphere at each site (note that field–blank SPMDs are typically left inside the open cans) during both deployment and retrieval of exposed SPMDs. The time period that field-blank SPMDs are exposed to site air should exactly mirror the time required to deploy and retrieve sample SPMDs. Afterwards, the cans with the field-blank SPMDs are resealed and shipped to the processing laboratory with the deployed SPMDs. Non-spiked (i.e., PRCs) field–blank SPMDs and PRC containing field-blank SPMDs are processed and analyzed exactly as deployed SPMDs. However, when perdeuterated PAHs are used as PRCs, then GC-MS, or capillary column (high resolution) or GC-FID must be used for separation and quantitation of native analytes and their perdeuterated analogs.

PRC Samples

When environmental conditions at an exposure site differ from laboratory calibration conditions or calibration data are not available, at least one SPMD per site is spiked with one or more PRCs (see “PERMEABILITY/PERFORMANCE REFERENCE COMPOUNDS [PRCs]”, page 3-31). The type of compounds used for PRCs and their spiking levels were discussed earlier. PRC samples and standard SPMD samples (i.e., field-deployed SPMDs) differ only by the presence of the PRCs. Handling, processing and analysis is also identical. As implied above, the purpose of the PRC sample is to provide data for estimation of the EAF or for *in situ* calibration.

SPMD Spikes

This type of QC sample (at least one per sample set) is used to determine the recoveries of target compounds from processed SPMDs and to establish "control limits" for the analytical process. The C.V.s for each analyte are used to set control limits for that compound. SPMD-fabrication or -process blanks are used for this type of QC sample. Sample processing and analysis of SPMD spikes is exactly the same as deployed samples. The triolein of individual SPMD blanks is directly fortified with target compound mixtures. The amounts of target compounds used for SPMD spikes (note that the carrier solvent volume should not exceed 10% of the lipid volume) are based on achieving an instrumental response that is representative of near the midpoints of the appropriate calibration curves. For example, 2 µg of each PP PAH is generally spiked into 1 mL of triolein in a standard SPMD. Assuming a 75% recovery, the concentration of each PP PAH will be 1.5 µg/mL (final sample volume of 1 mL), and upon analysis with GC-FID, the instrumental response will fall near the midpoint of the PAH calibration curve. In the case of OCs, 40 ng of each compound is generally spiked, and based on the same assumptions as given above, the concentration of each OC will be 30 ng/mL at a final volume of 1 mL. As in the example above, the GC-ECD response will fall at about the midpoint of the OC pesticide calibration curve.

Procedural Spikes

This type of spike is used (one for each sample set) when a rapid and independent assessment of individual steps in sample processing, and enrichment is desired. The spikes used are typically radiolabeled (i.e., ^{14}C or ^{3}H labeled) compounds, which normally consist of a high molecular weight PAH or a chlorinated compound. With the exception of the dialytic step, procedural spikes are directly injected into an appropriate solvent and are treated the same as sample extracts at the same point in the processing sequence. The

fortification levels used for these spikes are about the same as described earlier for the SPMD spikes. These QC samples are used as a trouble shooting aid to rapidly identify specific steps that contribute to low recoveries (i.e., out of control limits) of SPMD spikes and to develop control charts.

Expected QC Results

Based on the analysis of data from PCBs, OC pesticides and PAHs spiked into SPMDs (i.e., SPMD spikes), which have been subjected to the entire SPMD analytical procedure used at the CERC (see "PROCESSING AND RESIDUE ENRICHMENT", page 5-7 and "INSTRUMENTAL ANALYSIS", page 5-12), recoveries are generally > 75% with good precision, i.e., < 20% coefficient of variation (C.V.). Surprisingly, the C.V.s for the analysis of contaminants present in replicate samples (i.e., replicate SPMDs deployed contiguously at the same sites and treated identically during analysis) are often equivalent to or less than the C.V.s of SPMD spikes. This observation suggests that the variability of sampling rates of replicate SPMDs in the field is very small.

DeVita and Crunkilton (1998) have examined QC associated with the use of SPMDs. The results of their study showed that quality control measures applied to SPMD analysis met or surpassed conventional guidelines (EPA Method 610 for PAHs in water was used for this comparison) for precision and accuracy. This elevated level of QC was achieved even though measurements of both overall precision and accuracy of SPMD data encompassed more steps (each with the potential for variability) than the conventional method. In summary, DeVita and Crunkilton (1998) found that QC measures could be used to validate data from the analysis of SPMDs used in the field. In view of the state of SPMD QC, it appears that the SPMD approach for monitoring hydrophobic organic contaminants is equivalent to some EPA "approved" methods.

PROCESSING AND RESIDUE ENRICHMENT

The processing, enrichment, and fractionation of SPMDs have been described in a number of publications (Petty *et al*., 1995; Petty *et al.,* 1998; Huckins *et al*., 1996; Lebo *et al*., 1995; Bergqvist *et al*., 1998a; etc.). Processing, enrichment, and fractionation of SPMD samples generally involves the following steps: 1) removal of exterior surficial periphyton and debris, 2) organic solvent dialysis, 3) size exclusion chromatography, and 4) class-specific fractionation using Florisil®, silica gel and/or alumina adsorption chromatography. Figure 5-1, page 5-9 illustrates the generalized approach used. All solvents used in these procedures are of high purity.

Before initiating dialytic recovery of concentrated analytes, surficial material (i.e., periphyton, carbonate salts, etc.) on the SPMD membrane must be removed. First, each SPMD is rinsed in about 200 mL of hexane in a glass beaker for about 20 to 30 seconds and the hexane is discarded. Subsequently, the SPMDs are placed in a stainless steel pan and washed with copious amounts of running water (tap water is generally used but 1-L samples of the water used should be analyzed for any potential interferences) while being scrubbed vigorously with a clean toothbrush. At this point the SPMDs are examined for small holes in the membrane. If a hole is found, and other replicate SPMDs are not available, the hole is isolated by heat sealing. After the integrity of the SPMDs has been ensured, the SPMDs are submerged in a tank of 1-M HCl for approximately 30 seconds to remove any adhering mineral salts. Following the HCl treatment, the SPMDs are again rinsed with running water to remove the acid. All water on the membrane surfaces is removed by rinses of acetone, followed by isopropanol. The SPMDs are allowed to air dry for a minimal time period (typically < 6 minutes) on a piece of solvent rinsed aluminum foil.

Because of the very-low levels of interferences observed in SPMDs (Lebo *et al.,* 1995), individual devices can be combined to create a composite sample. This allows for lower detection and quantitation limits and provides increased contaminant mass for use in

bioassays or other endpoints. Dialytic recovery of analytes from intact SPMDs (Huckins *et al*., 1990b; Huckins *et al*., 1993) is conducted using glass jars fitted with solvent-rinsed aluminum foil under screw-type lids. (Note that the dialytic technique is also used for the cleanup of extracts from other environmental samples and it is the subject of several journal articles, which include Meadows *et al.* [1993], Strandberg *et al.* [1998], and Bergqvist *et al.* [1998a]). A minimum of 180 mL of high purity hexane per standard SPMD is used for dialysis. SPMDs are generally dialyzed individually. The procedure consists of an 18-h dialytic interval followed by a second, 4- to 6-h dialytic interval (note that analyte recovery differences between 4- and 6-h dialysis periods are insignificant) with fresh solvent. Dialytic separations are performed at 18 °C (constant), because this temperature has been shown to minimize the amount of co-dialyzed lipid components and LDPE oligomers, while maintaining good-to-excellent recoveries of analytes. The two dialysates for each sample are combined and quantitatively transferred to round bottom flasks. The dialysate volumes are reduced to approximately 5 mL using rotary evaporation, quantitatively transferred to test tubes by filtration through a pre-rinsed glass fiber filter, and subsequently reduced in volumes to approximately 1 mL.

The following liquid chromatographic systems are typical of that used at CERC for further enrichment and fractionation of analytes. The concentrated dialysate is subsequently subjected to size exclusion chromatography (SEC) to remove co-extracted lipid materials and PE oligomers. A typical SEC system consists of a Perkin- Elmer series 410 high performance liquid chromatograph, equipped with a Perkin-Elmer ISS-200 autosampler (Perkin-Elmer, Inc., Norwalk, CN), an ISCO Foxy 2000 fraction collector (ISCO, Inc., Lincoln, NE) and a 300 mm x 21.2 mm i.d.; 10 μm particle size, 100 Φm pore size Phenogel ® column (Phenomenex, Inc., Torrance, CA). Equivalent components and columns may be used for the SEC treatment. The mobile phase consists of 2% methanol in 98% dichloromethane. This step results in the elimination of nearly all lipid materials, PE oligomers, and elemental sulfur. The chromatography system should be calibrated

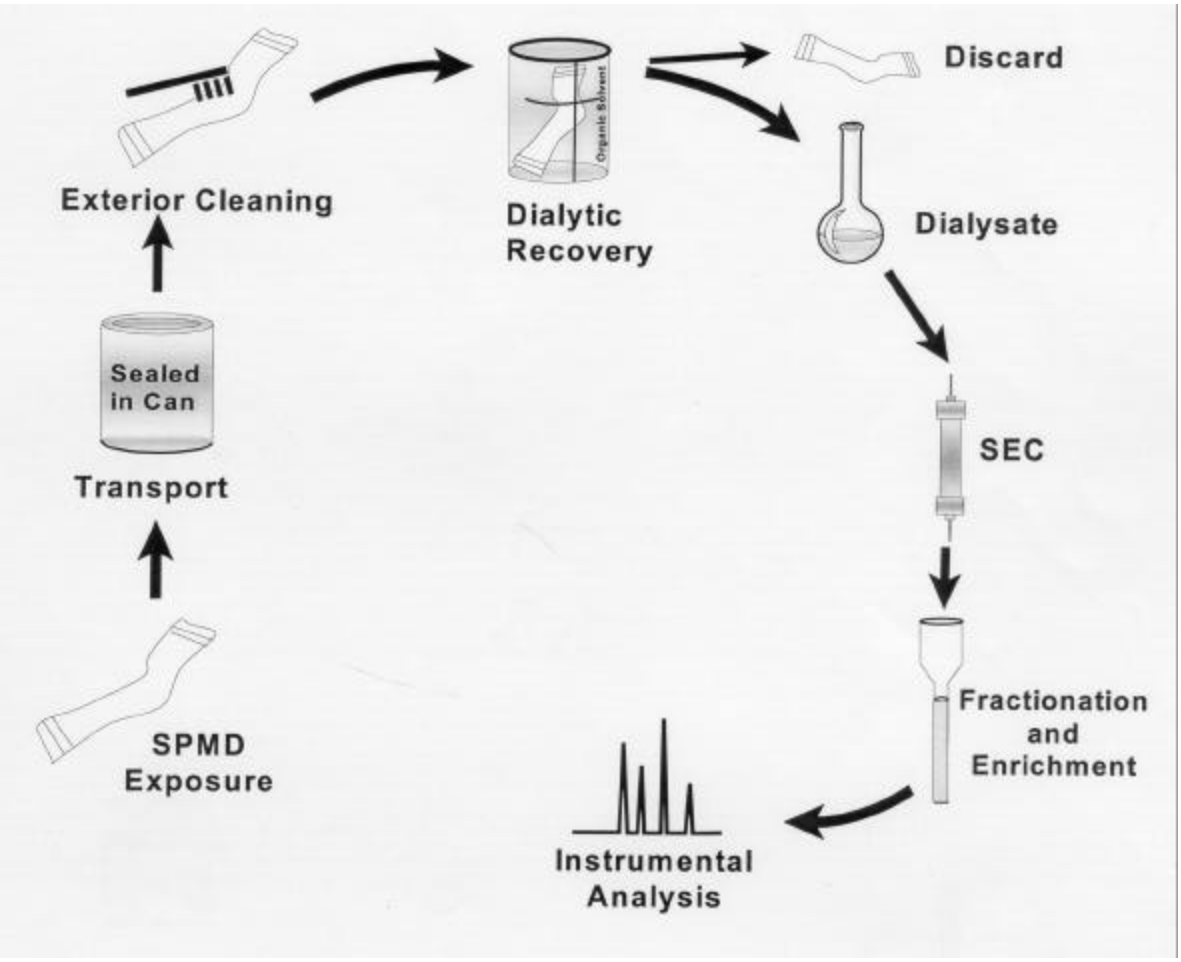

Figure 5-1. Key aspects of the SPMDs sampling and analysis process. *Often class fractionation is necessary after SEC when extracts contain complex mixtures of chemicals.*

on a daily basis. At CERC, this is accomplished by injecting a solution containing di-2-ethylhexylphthalate (DEHP), biphenyl, naphthalene, coronene, and elemental sulfur. These compounds elute in the order listed. Elution profiles are monitored using a UV detector (254 nm) and a strip chart recorder. The "collect" fraction is initiated between the apex of the DEHP peak and the biphenyl peak. More specifically, the cutoff between the dump and collect fraction should be after 70% of the time has elapsed between the apices of these two peaks. The end of the collect fraction should be after 70% of the time has elapsed

between the apices of the coronene and sulfur peaks. The collect fraction contains chemicals such as PAHs, PCBs, OCs, etc. This collect fraction is reduced in volume to approximately 1 mL using rotary evaporation and nitrogen (high purity) blow-down. The resulting concentrated extract is typically adjusted to a final volume of about 2 mL.

Chemical class fractionation and enrichment procedures vary from analytical laboratory to analytical laboratory. Thus, it is not possible to provide one overall method for fractionation prior to analysis. However, CERC's procedures are presented as a typical example. Following SEC treatment, the SPMD sample extracts are enriched using open column (glass) adsorption chromatography. Because different enrichment techniques are employed for PAHs than for PCBs and OCs, the sample extracts are split into two equal portions. The half designated for PCBs and OCs (1 mL), is applied to an activated Florisil (130 °C) column (5 g) and target compounds are eluted with 60 mL of 75:25 (V/V) methyl *tert*-butyl ether: hexane. Following volume reduction (1 mL), the eluate is applied to an activated silica gel (130 °C) column (5 g). Two fractions are collected; fraction SG-1 (46 mL of hexane) and SG-2 (55 mL of 40:60 [V/V] methyl tert-butyl ether: hexane). The PCB residues and several frequently found OC pesticides elute in SG-1, and the remaining OC residues elute in SG-2. The PAH designated half of each sample extract (1 mL) is treated using a tri-adsorbent column consisting of (top to bottom) 3 g phosphoric acid/silica gel; 3 g potassium silicate (KS); and 3 g of activated silica gel. The PAHs are eluted from this-tri-adsorbent column with 50 mL of 4% methyl tert-butyl ether in hexane.

Potential SPMD Specific Interferences

While the extracts of SPMDs are generally less difficult to purify than are extracts of tissue or sediment, certain interferences can be problematic for some types of analyses. The most important of these potential interferences are co-dialyzed polyethylene oligomers (i.e., the so-called polyethylene waxes), oleic acid, and methyl oleate. The latter two

interferences are residual from the synthesis of the triolein. Together these interferences are present in dialysates at < 30 mg per standard 1-mL triolein SPMD (see "SPECIFICATIONS OF THE STANDARD SPMD", page 4-3) or ≈ 6 mg/g of SPMD (Huckins *et al*., 1995a). Another potential interference is elemental sulfur, which is concentrated by SPMDs during some environmental exposures. However, both polyethylene waxes and elemental sulfur are readily removed using the aforementioned SEC procedure.

Unfortunately, small amounts of oleic acid and methyl oleate are generally present in the post-SEC treated sample extracts. Both of these lipids can be a source of interference when the concentrated SEC eluate is evaluated by GC-MS. However, the interference from oleic acid and methyl oleate is generally greater for SPMD field-blanks, fabrication blanks, and process blanks relative to environmentally exposed SPMDs. The lower level of interfering lipid in environmentally exposed SPMDs is due to the diffusion of a large portion of both methyl oleate and oleic acid to the exterior membrane surface (during exposures), where the residues dissipate or degrade.

Oleic acid can be completely removed by using the tri-adsorbent column, or more specifically, by using a small column of KS (5 g) and eluting with 50 mL of a 1:1 (V/V) mixture of hexane and dichloromethane. Also, the previously described Florisil column removes any residual oleic acid in the post-SEC extracts. However, the removal of residual methyl oleate is often more problematic. While most of the methyl oleate is eliminated during the aforementioned SEC treatment, a small portion remains in the sample extract. Because the methyl oleate contains a polar functional group, it is found in the SG-2 fraction rather than SG-1. Methyl oleate causes little or no problem when the analysis is performed using GC-ECD or GC-PID. However, it interferes with GC-FID determination of PAHs or full scan GC-MS analyses. If necessary, methyl oleate concentrations can be further reduced (≈ 99.6% reduction) by another pass through SEC.

Also, residual methyl oleate can be completely removed using destructive techniques, such as cleanup with sulfuric acid impregnated silica gel. Unfortunately, this approach is only applicable for compounds that do not degrade in the presence of strong acid (e.g., PCBs, chlorinated dioxins and furans, and selected organochlorine pesticides).

Recently, Gustavson and Harkin (2000b) developed a convenient and novel solid phase extraction (SPE) method for the removal of methyl oleate from SPMD dialysates containing PAHs. A small SPE tube (1-g or 0.5-g) containing a dual-zone silica (normal phase)-based restricted-access sorbent (Diazem, Midland, MI, USA) is used for the separation. The capacity of this sorbent to remove methyl oleate is ≈1.8% (lipid/sorbent, w/w). The PAHs are eluted with 19 mL of hexane and methylene chloride (97:3, V/V) and recoveries of all PAHs were ≥ 72%. Finally, Lebo *et al.* (2000) has recently developed a promising method for the removal of methyl oleate from triolein, prior to its use in SPMDs.

INSTRUMENTAL ANALYSIS

The SPMD dialysate can be analyzed directly (e.g., high performance liquid chromatography [HPLC]), after SEC cleanup, or after adsorbent chromatography. A wide variety of instrumental techniques can be employed. Among these techniques are HPLC, GC, and GC/MS, etc. In fact, any analytical technique used for determining the presence and concentrations of chemicals in environmental matrices can be applied to the analysis of chemicals in SPMD extracts. The types and levels of chemicals expected to be present in the SPMD extract will dictate the analytical procedure to be employed.

Typical examples of GC methods applied at CERC for the analysis of OCs, PCBs, and PAHs are presented for illustrative purposes. The individual investigator determines the choice of instrumentation and the exact method. In our case, we use a Hewlett Packard 5890 series GC or equivalent, equipped with a Hewlett Packard 7673A autosampler or

equivalent (Hewlett Packard, Inc., Palo Alto, CA). In these analyses, 1.0 µL of sample extract is injected using the "cool, on-column" technique with hydrogen as the carrier gas. Analyses of the SG-2 fraction (OCs) and the SG-1 fraction (OCs and PCBs) are performed using a DB-35MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) capillary column (J&W Scientific, Folsom, CA) with the following temperature program: injection at 90 ^{o}C; then 15 ^{o}C/min to 165 ^{o}C; followed by 2.5 ^{o}C/min to 250 ^{o}C; then at 10 ^{o}C/min to 320 ^{o}C. The electron capture detector (ECD, Hewlett Packard, Inc., Palo Alto, CA) is maintained at 330 ^{0}C. Quantitation of OCs in SG-1 and in SG-2 is accomplished using six-point calibration curves with octachloronaphthalene (OCN) as the instrumental internal standard. The levels of the OC standards span a 80-fold range of concentration for each compound determined. Quantitation of total PCBs in SG-1 is accomplished using a six-point curve employing solutions containing a 1:1:1:1 mixture of Aroclor® 1242, 1248, 1254, and 1260, with OCN as the instrumental internal standard. The levels of the PCB standards span a 40-fold concentration range.

Analysis of PAH fractions is performed using a DB-5 (30 m x 0.25 mm i.d. x 0.25 µm film thickness) capillary column (J&W Scientific, Folsom, CA) with the following temperature program: injection at 60 ^{o}C, then 15 ^{o}C/min to 165 ^{o}C, followed by 2.5 ^{o}C/min to 250 ^{o}C, then 10 ^{o}C/min to 320 ^{o}C and held at 320 ^{o}C for 1 min. Detection is performed using a Hewlett Packard flame ionization detector (FID) or a GC-MS system. Quantitation of PAHs is accomplished using a six-point curve with D_{14}-4-Terphenyl as the instrumental internal standard. The levels of the PAH standards span a 32-fold range of concentration for each priority pollutant PAH.

Due to the complexity of the mixture of chemicals often sequestered by SPMDs deployed in the field, it may be necessary to employ GC/MS or HPLC/MS to confirm the presence of analytes of interest and to tentatively identify unknown chemicals. Several examples serve to illustrate. SPMDs deployed in the Missouri River in 1994 (Petty *et al*., 1998) were

found to contain quantifiable levels of OCs, PCBs, and PAHs. In addition, an examination of the purified SPMD extract using GC/MS with negative chemical ionization revealed the presence of more than 40 additional chemicals. Many of these chemicals, while not unequivocally identified, were determined to contain both chlorine and bromine as constituents. In another example, a purified SPMD extract from a deployment in the Las Vegas Wash area in 1995, was examined using the same technique and contained more than 100 individual components (Leiker, 1998). In essence, using SPMDs provides an approach for determining not only the presence of recognized contaminants, but also a means of defining the presence of unknown chemicals.

DATA FORMAT AND COMPARABILITY

In the literature (see "SOURCES OF SPMD INFORMATION", "Appendix A" page A-1), SPMD data have been reported in a variety of ways. The concentrations of chemicals in the devices are given as pg or ng per g of lipid or per g of whole SPMD (i.e., lipid plus membrane). If an investigator chooses to report SPMD levels on the basis of lipid mass then the lipid phase must be measured separately, or if the whole SPMD is analyzed using dialysis for analyte recovery, the K_{mL} partition coefficient must be known to permit estimation of the mass fraction of chemical in the lipid. In this case the following model is used

$$C_L = M_{AD} / (M_L + K_{mL} M_M) \qquad \text{(Equation 5-1)}$$

where M_{AD} is amount of analyte in the dialysate and M_L and M_M is the mass of the lipid and membrane phases, respectively. When using C_L to estimate C_W it is important to realize that k_us and R_ss are based on the whole SPMD and a lipid-weighted sampling rate would be needed for the derivation of ambient water concentrations.

SPMD concentrations are frequently compared with those found in biota from the same sample location. Often lipid-normalized SPMD and biota data are compared, which is only appropriate when both matrices have attained steady state or equilibrium. This principle is illustrated in Figure 5-2, page 5-16, where significant errors are shown to occur for samples still in the uptake kinetics phase, and the assumptions and requirements of the approach are examined. The first-order rate constants (i.e., k_us) for uptake by SPMDs and biomonitoring organisms provide the best comparison of the two matrices, when steady state concentrations have not been attained. Also, comparison of the total mass of analyte sequestered per g of whole body tissue and per g of whole device, relative to the concentrations of interferences in the two matrices, is informative. Unlike SPMDs, biomonitoring organism mass can be expressed in terms of wet and dry weights, which complicates comparisons of the two approaches. Actually, this potential problem also exists when comparing fish and bivalves, because fish concentrations are typically expressed on a wet basis, while bivalves are usually given in terms of dry weight. Where possible it is preferable to compare wet weight concentrations of tissues to whole SPMD concentration. If dry tissue weights must be used (often the variance associated with wet weight determinations is high), then a comparison of the total masses of analytes concentrated in the two sample matrices should be included to help prevent misinterpretation of relative sampling efficiency.

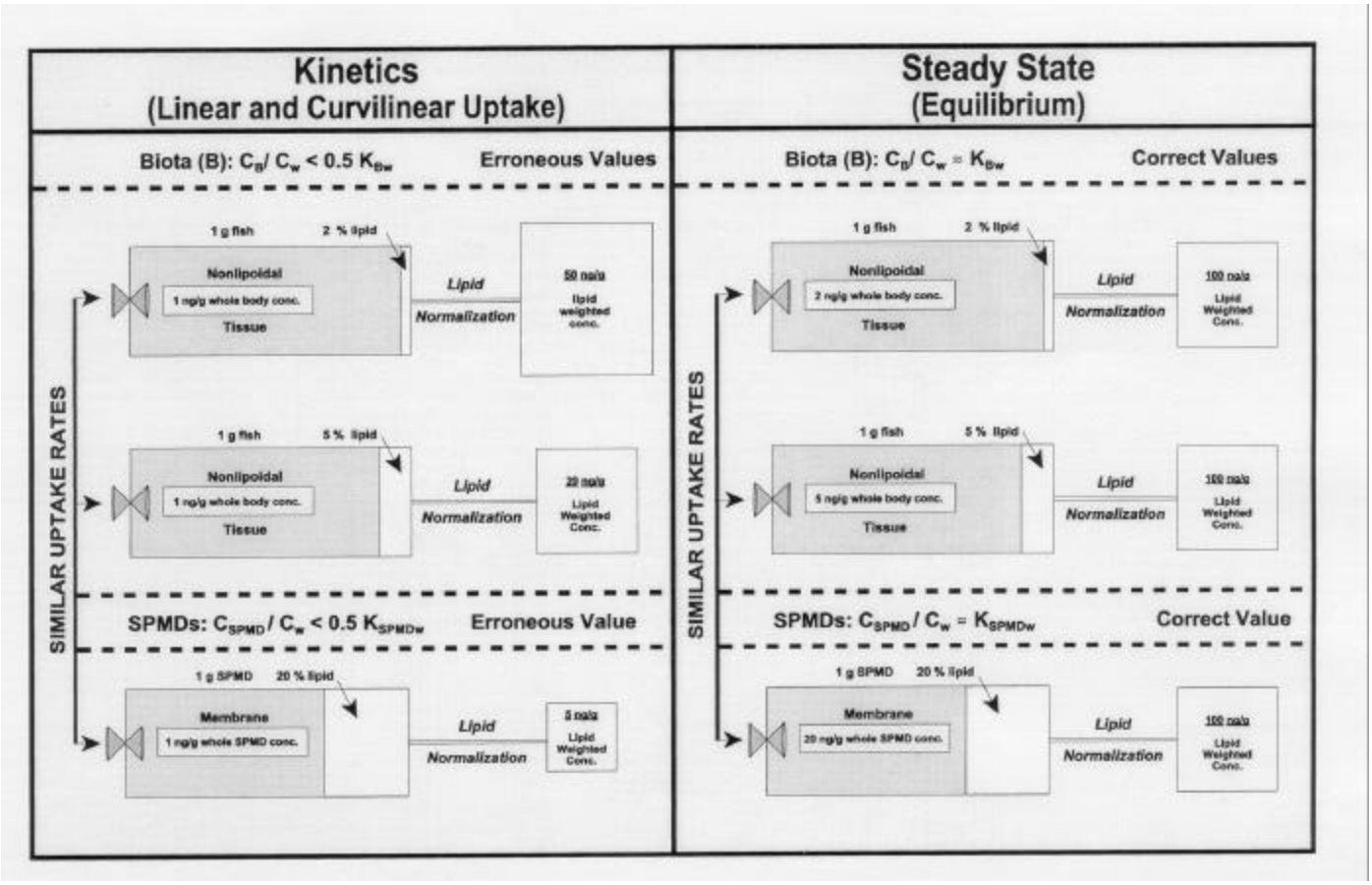

Figure 5-2. Illustration of potential problems associated with lipid normalization of SPMD and biota chemical concentrations: kinetics versus equilibrium.

Section 6

BIOASSAY OF SPMD EXTRACTS OR DILUENTS

In addition to instrumental methods of analysis, the complex mixtures of chemicals often sequestered by SPMDs are amenable to examination by a variety of bioassays (Huckins *et al*., 1996; Zajicek *et al*., 1996; Parrott and Tillitt, 1997; Johnson, 1998; Petty *et al*., 1998; Parrott *et al*., 1999; Sabaliunas *et al*., 2000a; Sabaliunas *et al*., 2000b). These assays include biomarker/bioindicator tests, immunoassays, and classic toxicity tests. Bioassays used to assess SPMD extracts or diluents include the following: Microtox®, Mutatox®, mixed function oxygenase (MFO) induction-Ethoxyresorufin-O-deethylase (EROD) activity, sister chromatid exchange (SCE), vitellogenin (VGT) induction via interperitoneal injection of test species, enzyme-linked immunosorbent assay (ELISA), Daphtoxkit F®, and Ames mutagenicity test (note that this list is not all inclusive). Some of these assays incorporate metabolic activation systems (e.g., Mutatox and EROD). This approach makes it possible to account for metabolic transformation processes that may enhance or reduce the effects of chemical residues in organism tissues. The marriage of SPMDs and compatible bioassays offers many avenues of investigation, all potentially providing information concerning the relative toxicological significance of chemicals present in the environmental matrices sampled.

A major challenge for ecotoxicologists is to obtain samples from environmental systems that are suitable for testing. Most hydrophobic organic contaminants are present in environmental waters only at trace levels (i.e., < 1 µg/L). However, the sometimes-slow process of bioconcentration (uptake from water)/bioaccumulation (uptake from both water and diet) can lead to elevated concentrations of contaminants in aquatic organisms, which can result in a variety of adverse effects. These chemical uptake processes are especially relevant to the magnitude of environmental impacts of persistent hydrophobic organic contaminants and those with log K_{ow}s in the range of 4.0 to 7.0 are of particular concern. In

many cases, bioassays (especially the more rapid cellular-based assays) do not account for the potential effects of bioconcentration. Also, a number of the aforementioned assays have relatively low sensitivities for many common pollutants. For example, the Microtox test often requires high ng to low µg amounts of priority pollutants to elicit a measurable response. Thus, direct testing of environmental waters with this bioassay may lead to false-negative errors in assessing the potential risk of waterborne residues to aquatic life. To avoid this type of error and expand the use of biomarker tests for ranking toxicity potential, a preconcentration method is needed that mimics the bioconcentration process. SPMDs offer several advantages over other potential preconcentration methods that include: 1) a mimetic (defined earlier) design, 2) only bioavailable dissolved-phase residues are sampled, 3) sampling is generally integrative, which permits the sequestration of episodic contamination events, and 4) significant statistical advantages, due to high reproducibility, relative to biomonitors (Prest *et al*., 1997; Huckins *et al*., 1998a). Consequently, the SPMD-bioassay assessment approach should enhance an investigators ability to screen for the toxicological significance of bioconcentratable environmental residues.

If an investigator demonstrates that an SPMD concentrate is toxic or genotoxic, when using a specific biomarker test, questions may arise as to the relevance of the finding in regard to risk assessment. Clearly, the SPMD-biomarker/bioassay combination is useful as a screening tool for ranking the potential toxicities of bioconcentratable residues at multiple sites and for determining sources of pollutants. However, the justification for a specific level of preconcentration to account for the toxicological effects of residue bioconcentration in tissues is less clear. This is, in part, due to the wide variation in BCFs for the same chemical among different species. In addition, depending on the specifics of biomarker experimental conditions (e.g., exposure or incubation time) and the type of organisms or cell lines used, chemicals in the incubation medium are also concentrated by the test organisms to some unknown degree. Note that significant bioconcentration is unlikely in the case of the basic Microtox test, because the exposure time is only 5 min.

Regardless of the complexity of predicting an appropriate preconcentration factor, some level of preconcentration is needed to permit application of biomarker/bioindicator tests and other rapid bioassays to trace aquatic contaminants. Obviously, the maximum level of preconcentration or the SPMD CF should not exceed the measured or estimated BCF of a chemical for the species of concern. However, this may not be true when some organisms metabolize compounds of concern (clearly, metabolic activity differences among species affect residue body burdens [Connell, 1990]) and others do not. Currently, the SPMD exposure duration, and thus analyte CF, is operationally defined by the investigator but typically should not exceed 30 days (EROD for ultra-trace levels of dioxins, furans and coplanar PCBs may be an exception). Exposures of 30 days or less generally result in SPMD CF less than the BCFs of many stable hydrophobic compounds in test organisms.

Extracts or lipid rinses from exposed SPMDs contain chemicals present in the whole or a fractional volume of the sampler. After transferring the enriched SPMD extract or diluent (lipid rinse) to an appropriate carrier solvent, aliquots are used for assays (see “POTENTIAL INTERFERENCES”, page 6-12, for potential biomarker interferences). If the test endpoint is an EC_{50} (e.g., mg or g of sampler matrix/mL or L of carrier solvent), then this concentration value represents a specific mass of SPMD lipid or whole SPMD, per unit volume carrier solvent, that elicits a toxic response. Obviously, investigators must keep track of sample splits, dilutions, etc. When analytical chemistry is performed and SPMD calibration data are available for detected residues, the measured EC_{50} value can be related back to a specific volume of water ($V_{w\text{-}tox}$) at a sample site, which contained sufficient mass of bioavailable contaminants to elicit the observed toxicological response. If the above conditions are met the following model can be used to derive $V_{w\text{-}tox}$

$$V_{w\text{-}tox} = k_u \, t \, EC_{50} \, V_c \qquad \text{(Equation 6-1)}$$

Where the group $k_u t$ represents the volume of water extracted by the SPMD, and V_c is the volume of carrier solvent used in each determination of the EC_{50} value. If k_us are available for the chemicals identified in the SPMDs and for the aquatic organisms of interest (e.g., see Mackay *et al*., 1992b; Mackay *et al*., 1992a; Mackay *et al*., 1997), then $V_{w\text{-}tox}\ C_w$ (represents mass of bioavailable contaminants eliciting the biomarker response) can be compared to organism $k_u\ t\ M_o\ C_w$ (mass of bioavailable contaminants exposed to organism of concern during a specified time interval [t]); where M_o is organism mass in g. Obviously, this approach is limited by a paucity of rate constant data, the potential for unidentified contaminants to elicit toxic responses, and the lack of data on chemical concentrations in biomarker cell lines or organisms (i.e., the CF associated with the transfer of chemicals from the test medium to the test cells or organisms).

Figure 6-1, page 6-5 illustrates the level of SPMD processing/enrichment generally used in preparing samples for testing with bioassays/immunoassays. The following are brief descriptions of several commonly used biomarkers/bioassays, which have been used in conjunction with SPMDs. However, it is beyond the scope of this guide to discuss all bioassays that may be successfully used in conjunction with SPMDs

MICROTOX AND MUTATOX

Several investigators (e.g., Huckins *et al*., 1996; Cleveland, *et al*., 1997; Johnson, 1998; Sabaliunas *et al*., 2000a; Sabaliunas *et al*., 2000b) have determined the toxicity and genotoxicity (i.e., DNA-damaging potential) of purified SPMD extracts, or SPMD lipid diluents, using the Microtox and Mutatox assays (AZUR Environmental, Carlsbad, CA), respectively. These related *in vitro* tests are based on chemically induced changes in the level of light generated by bioluminescent *Photobacterium/Vibrio* (Microtox) and a dark mutant strain of *Photobacterium/Vibrio* (Mutatox). The degree of the decrease in light (Microtox) or increase in light (Mutatox), when compared to controls, indicates the

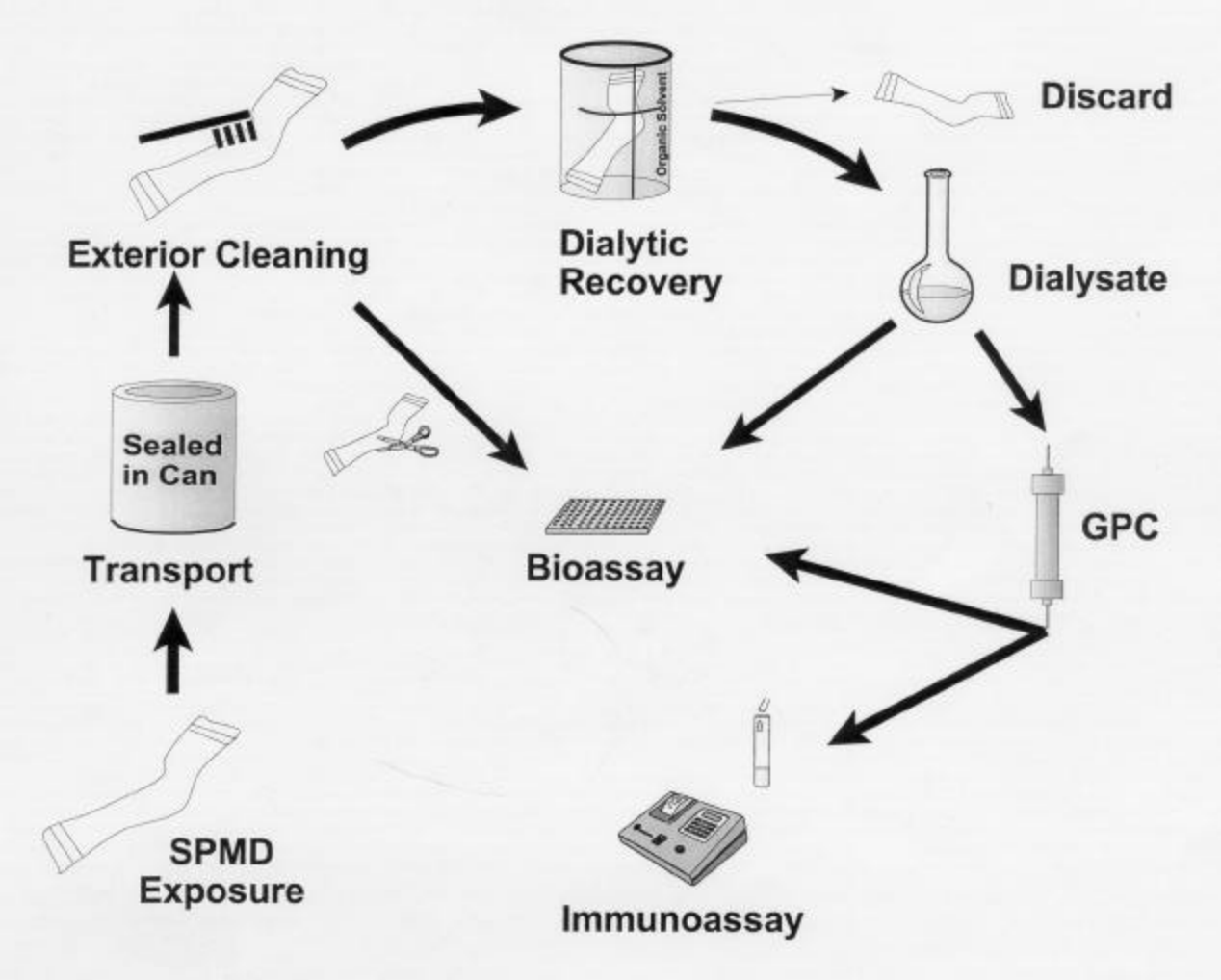

Figure 6-1. Illustration of SPMD *in vitro* bioassay and immunoassay protocol, which includes sampling, processing, and bioassay or immunoassay.

relative acute toxicity (i.e., the basic Microtox test) and genotoxicity (Mutatox) of the sample extract, respectively. The toxicological endpoint for the Microtox test is an EC_{50} value and 95% confidence interval (i.e., the test is quantitative), whereas the endpoint for the Mutatox test is qualitative, providing a yes or no assessment of the presence of DNA-damaging substances. Johnson (1998) has determined the acute toxicity and genotoxicity of many chemicals, including PAHs (see Table 6-1, page 6-7). Table 6-2 (page 6-8) gives an

example of using Microtox and Mutatox to determine the potential toxicity of SPMD extracts in two separate studies (Huckins *et al.,* 1996). Note that the positive Mutatox response for the Flat Branch sample (Table 6-2, page 6-8) is not surprising because of the relatively high levels of PAHs detected there.

Microtox responds well to a wide array of hydrophobic chemicals (see the work of Johnson, 1998 in which a variety of pesticides, industrial chemicals and petroleum products are tested). This is apparently due to the response of Microtox to compounds with a narcosis mode of toxicity (Johnson, 1998; Sabaliunas *et al.*, 1998), which is nonspecific. As shown by Johnson (1998) chemicals that elicit narcosis include multiple chemical classes.

Clearly, the mode(s) of action eliciting a genotoxicity response is more chemical-structure specific (Johnson, 1998). As suggested earlier, *in vitro* metabolic activation is required to assess the genotoxicity of SPMD residues with Mutatox. Typically, a rat liver S9 fraction is used for the exogenous metabolic activation step (AZUR, Inc., 1992). At first glance, Mutatox appears to be well suited for the assessment of SPMD extracts. However, Sabaliunas *et al*. (2000b) has pointed out several potential difficulties/ shortcomings of the test in its present form. These include reduced light intensity due to cytotoxicity or cell death, delays in the genotoxic response of some samples beyond standard measurement times, and lower sensitivity of measurements based on reverse mutations. Test sensitivity is not an issue when SPMDs are used to preconcentrate samples. Also, in the Mutatox protocol (Johnson, 1998), a sample is designated as genotoxic only when two positive responses are recorded at different concentrations in a single dilution series. Generally, cytotoxic effects should be evident from the shape of the dose-response curve, and increased turbidity in exposed samples relative to controls is also used as an cytotoxicity indicator (Johnson, 1998). However, the concerns raised by Sabaliunas *et al*. (2000a), require further investigation before Mutatox can be used with confidence.

Table 6-1. Toxicological Evaluation of Polyaromatic Hydrocarbons (PAHs with Microtox Basic Test and Mutatox [a].)

Compounds	Microtox		Mutatox
	EC_{50} [b]	CI [c]	Genotoxicity [d]
acenaphthylene	0.34	0.25-0.47	Positive
phenanthrene	0.48	0.33-0.68	Positive
fluorene	0.50	0.35-0.70	Positive
anthracene	0.64	0.53-0.78	Positive
benz[a]anthracene	0.73	0.65-0.81	Positive
acenaphthene	0.75	0.69-0.81	Positive
2-aminoanthracene	0.75	0.49-1.2	Positive
fluoranthene	0.83	0.63-1.08	Positive
naphthalene	0.90	0.85-0.99	Positive
chrysene	0.92	0.85-0.99	Positive
2-aminonaphthalene	1.3	1.1-1.5	Positive
2-acetamidofluorene	2.3	1.3-4.1	Positive
2-aminofluorene	4.1	2.5-6.4	Positive
benzo[a]pyrene	10.7	6.4-18.2	Positive
3-methylcholanthracene	19.9	18.3-21.5	Positive
7,12-dimethylbenzanthracene	33.1	14.6-74.7	Positive
pyrene	>500	---	Positive
DMSO (Control)	ND [e]	---	Negative

[a] Data from Johnson (1998).
[b] 5 minute EC_{50} = µg/mL.
[c] CI = 95% confidence interval.
[d] 1% rat S9 activation.
[e] ND = not detected.

Table 6-2. Use of Microtox and Mutatox to Determine the Toxicity of SPMD Concentrates. Microtox values are 5-minute EC_{50}s with 95% confidence intervals (in parentheses).

Sample Type	Microtox Toxicity EC_{50} [a]	Mutatox Genotoxicity
SPMDs		
Winter Quarters Bay [b]	3.1 (2.9 – 3.3)	Negative
McMurdo Sound [b]	88 (28 – 275)	Negative
Flat Branch [c]	NA [d]	Positive
Quality Control		
Procedural Blank [e]	ND [f]	Negative
Laboratory Blank SPMD [g]	ND	Negative
Microtox Phenol Reference Toxicant (µg/mL H_2O)	19 (17 – 21)	NA
Mutatox Benzo[a]pyrene Reference Toxicant (1.0 (µg / Vial)	NA	Positive

[a] Assays were conducted on lipid diluent or dialysates and EC_{50} values represent mg SPMD lipid/mL carrier solvent.
[b] SPMDs exposed to Antarctica sediments in microcosms (Huckins *et al.,* 1996).
[c] SPMDs exposed to a small urban stream (Huckins *et al.,* 1996).
[d] None analyzed.
[e] Solvents and reagents used in tests.
[f] None detected.
[g] Freshly prepared Blank SPMD, carried through Microtox and Mutatox test.

MFO-EROD

SPMDs are often applied to concentrate trace levels of environmental contaminants that induce MFO activity (Huckins *et al*., 1996; Parrott and Tillitt, 1997; Parrott *et al*., 1999). MFOs are a group of enzymes that aid in the metabolism and clearance of many

hydrophobic compounds. One of the most widely recognized MFO enzymes is cytochrome P4501A1 (often measured catalytically as EROD activity). Increased levels of EROD activity indicate exposure to certain types of organic compounds. These include planar polycyclic aromatic compounds, either halogenated (e.g., polychlorinated dioxins and furans and planar PCBs) or PAHs. Because determination of EROD activity is relatively simple (Parrott *et al*., 1999), this endpoint is often used as a screening tool for the assessment of sites contaminated with the above classes of chemicals. H4IIE rat hepatoma cells and the PLHC-1 fish hepatoma cells (P*oecilieopsis lucida*) are the cell lines most commonly used for the measurement of EROD activity (Tillitt *et al*., 1991; Lebo *et al*., 1995; Huckins *et al*., 1996; Parrott and Tillitt, 1997). Enzymatic activity is standardized to cellular protein content using the method of Lorenzen and Kennedy (1993). This approach corrects for the attenuation of EROD due to cytotoxicity.

EROD activity is measured in the H4IIE cells as follows. The cells are seeded at 7,000 cells/ well in 250 µL of Dulbecco's modified Eagles culture media (Tillitt *et al*., 1991). After an initial incubation period of 24 h, the cells are dosed with 5 µL (isooctane carrier) volumes of enriched SPMD extracts (at least through SEC) and incubated for an additional 72 h. Sample dose is typically expressed as g-equivalents triolein or whole SPMD/mg cellular protein. Multiple exposures are performed at each of six (typically) sample concentrations, using a dilution series. Afterwards, the microtiter plates are washed three times with distilled water and the cells are lysed. EROD activity (pmol/mg cellular protein/min) in each sample is measured kinetically, and the linear portion of the sample dose-response curve is compared to a 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) standard response (pg TCDD/mg cellular protein) curve. The standard response curve is generally based on eight concentrations of TCDD, and it is used to quantify the total toxic equivalents (TEQs) of samples (Gale *et al*., 2000). TEQ values of samples represent the concentrations of TCDD required to give equivalent EROD responses. Ankley *et al.* (1991) have given details of the procedure for TEQ calculation.

Figure 6-2 , page 11 shows a specific example of the use of the H4IIE-EROD assay for purified SPMD extracts. After purification of SPMD extracts, Gale *et al.* (2000) showed that H4IIE determined TEFs were correlated to instrumentally determined TEFs.

OVERVIEW OF ADDITIONAL ASSAYS

As suggested earlier, a number of other assay have been successfully used with SPMDs, which include an immunoassay for PCBs, the Daphtoxkit F for insecticides, and the measurement of contaminant induced vitellogenin. Zajicek *et al.* (1996) explored the use of a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit from Ohmicron Corporation (the Ohmicron PCB RaPID Assay) to analyze PCB residues sequestered in SPMDs. He found a positive correlation (i.e., $R^2 = 0.999$, n = 3) between the PCB concentrations in SPMDs measured by the ELISA and PCBs measured by GC-ECD. ELISA kits are currently available for a number of types of contaminants, determination of test results is rapid, and the kits are generally inexpensive Petty *et al.* (1998) used a vitellogenin assay to assess the endocrine disrupting potential of contaminants in enriched SPMD extracts. Vitellogenin is an egg yolk phosphoprotein precursor, which is synthesized in the liver of female telosts in response to estrogen from the ovary (Bailey, 1957). A wide variety of environmental contaminants have been shown to have estrogenic activity (Colborn *et al*., 1993). Equal portions of enriched extracts from SPMDs, deployed in the Missouri River after the flood of 1993, were injected into immature rainbow trout (*Oncorhynchus mykiss*). The SPMD extracts contained elevated levels of complex contaminant mixtures, which included PAHs and pesticides. Three of the four fish injected with this sample exhibited vitellogenin induction, while no induction was observed in fish injected with SPMD process blanks.

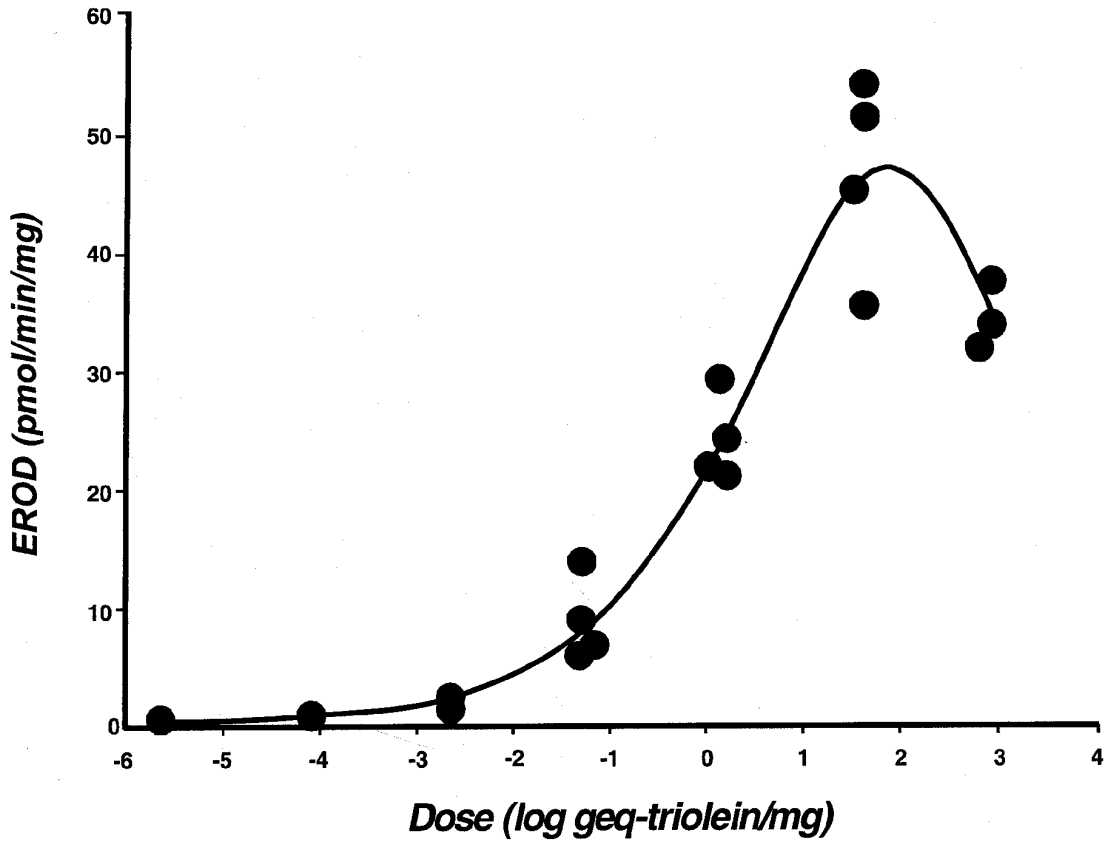

Figure 6-2. Ethoxyresorufin O-deethylase (EROD) induction in H4IIE rat hepatoma Cells exposed to an SPMD dialysate. ***Four Standard SPMDs were deployed for a 28-day period in Bayou Meto, Arkansas. Doses of dialysate were normalized to gram-equivalents of triolein per mg of cellular protein. Courtesy of Don Tillitt, CERC.***

Sabaliunas *et al.* (2000b) showed that a *Daphnia pulex* immobilization test (Daphtoxkit F) was far more sensitive to a mixture of insecticides sequestered in SPMDs than Microtox. This is not surprising because the OC and pyrethroid pesticides present in the enriched SPMD extracts are neurotoxins, and the effect thresholds can be much lower than narcosis-type toxicants. Thus, if insecticides are the contaminants of concern, this approach may have some advantage over Microtox.

POTENTIAL INTERFERENCES

The use of the above assays to evaluate the toxicity of SPMD extracts is not without potential interferences. Sabaliunas *et al.* (2000a) and Sabaliunas *et al.* (2000b) have examined the potential role of oleic acid and elemental sulfur as contributors to the toxicity of extracts from environmentally exposed SPMDs. The toxicity of fatty acids has been attributed to their membrane disturbing properties, which include disruption of the calcium pump by the formation of metal salts (Ewald and Sundin, 1993). As mentioned earlier, oleic acid is an impurity in the 95% triolein used in SPMDs, and may also be produced by biotic or abiotic hydrolysis of methyl oleate and triolein (note that this has not been demonstrated to occur during SPMD exposures). During environmental exposures, a significant portion of this triolein impurity diffuses to the exterior surface of an SPMD, where dissipation and/or degradation occur. Unfortunately no or little attenuation occurs in the oleic acid levels in laboratory SPMD-field blanks, -fabrication blanks and -process blanks. Thus the potential for a differential response exists among field exposed SPMDs and associated QC SPMD samples.

In the case of sulfur, hydrophobic elemental sulfur is taken up by bacterial cells and may be reduced to toxic sulfides (Brouwer and Murphy, 1995). Many types of sediment contain relatively large amounts of elemental sulfur and elemental sulfur is readily accumulated by SPMDs. However, a number of analytical methods can be used to remove these potential interferences from SPMD extracts. These include SEC, as described in the "SPMD ANALYTICAL CHEMISTRY" section, page 5-1 (both oleic acid and sulfur), shinny copper wool (sulfur only, see Petty *et al*., 1995), and KS (oleic acid). Note that other cleanup techniques are also available for these interferences, especially for oleic acid. Using a preemptive approach, Lebo *et al.* (2000) have shown that oleic acid can be removed from triolein, prior to use in SPMDs. Also, some biomarker tests have provisions for accounting for (e.g., EROD assays as described in this section) or detecting cytotoxicity (e.g.,

Microtox and Mutatox). Finally, the use of pyrogen-free carrier solvents that are compatible with the assays used are a prerequisite to good laboratory practices.

In conclusion, combining the power of SPMDs to concentrate the bioavailable portion of complex mixtures of chemicals present in aquatic environments with selected biomarker tests appears to provide a useful screening approach for determining the toxic potential of bioconcentratable aquatic contaminants. Methods have been published, such as Johnson *et al.* (2000) that provide guidance on the assessment of the toxicity of SPMD concentrates. Also, if contaminant identities are suspected or known, it may be feasible to use certain ELISA kits and EROD to estimate the concentrations of specific analytes in enriched SPMD dialysates.

Section 7

COMPARISIONS TO BIOMONITORING ORGANISMS

Side-by-side comparisons of SPMDs with biomonitoring organisms have become increasingly common in the literature (Prest *et al.*, 1992; Prest *et al.,* 1995a; Prest *et al.,* 1995b; Prest *et al.,* 1997; Ellis *et al*., 1995; Herve *et al*., 1995; Kolok *et al*., 1996; Huckins *et al*., 1996; Peven *et al*., 1996; Gale *et al*., 1997; Hofelt and Shea, 1997; Moring and Rose, 1997; Meadows *et al*., 1998, Sabaliunas *et al*., 1998; Wang *et al*., 1998; Huckins *et al*., 1998b; Axelman *et al*., 1999; Röe *et al*., 2000b). This list of citations is not all-inclusive, and there are numerous unpublished works as well. All of the above comparisons were restricted to delineating similarities and differences in the uptake and accumulation of organic contaminants, i.e., evaluation of SPMD-biomarkers versus direct use of biomonitoring organisms was not explored.

Based on the results of the above studies, there is a need for a summary of current findings on the similarities, differences, advantages and disadvantages related to the use of SPMDs and aquatic organisms for monitoring organic contaminants in water. Also, we briefly examine theory and mechanisms, related to the accumulation of hydrophobic organic compounds in these matrices, to aid in our evaluation of these issues. For a more in-depth review on how bioconcentration/bioaccumulation occurs, and the variables mediating these phenomena, see Connell, 1990 and Huckins *et al*., 1997c. Note that a discussion on the format of residue concentration data for appropriate comparisons of SPMDs and biomonitoring organisms was presented earlier under “DATA FORMAT AND COMPARABILITY”, page 5-14. Also, see “EXAMPLES OF APPLICATIONS”, page B-1, for several of side-by-side comparisons of SPMDs and biomonitoring organisms.

Cleary the physical differences between an inanimate, passive sampling device, such as an SPMD, and living organisms are vast. However, in regard to the uptake of chemicals from water, we need only focus on structural features and mechanisms that mediate the concentration of neutral organic contaminants in the two matrices. Similarities among SPMDs and biomonitoring organisms include the following: 1) SPMDs and aquatic organisms concentrate hydrophobic chemicals from water via the processes of passive diffusion and partitioning between lipids and the lipid like membrane (SPMD) and water, 2) the triolein used in SPMDs is a major lipid in fishes (Huckins *et al*., 1990a) and is representative of the neutral lipid class (Chiou, 1985), where persistent hydrophobic contaminants are stored in fish tissues, 3) resistance to mass transfer across the aqueous boundary layer associated with fish gills and the SPMD membrane is likely the rate-limiting-step for many hydrophobic compounds, 4) side-by-side comparisons of finfishes and shellfishes with SPMDs indicate that the two matrices generally accumulate the same types of organic compounds, and that homologue concentration patterns and uptake rates are relatively similar (Peven *et al*., 1996; Hofelt and Shea, 1997; Gale *et al*., 1997; Meadows *et al.,* 1998; Prest *et al*., 1997; Röe *et al*., 2000b). Obviously, there are major exceptions to statement "4", which will be subsequently discussed.

The best correlation between the uptake of chemicals by finfish or bivalves and SPMDs is expected at the stage of chemical transport and absorption across the blood-water interface (i.e., the gills) of aquatic organisms. During the steady-state phase (i.e., linear uptake kinetics) of biouptake, the types and quantities of storage lipids and the potential *in vivo* biotransformation of analyte residues should play a minimal role. Figure 7-1 shows that a plot of the PP PAH uptake rates by SPMDs has a parabolic shape similar to a plot of organic chemical uptake across trout gills (McKim *et al.,* 1985*)*. This illustration lends credence to the hypothesis that the resistance or rate-limiting step of residue transport across the blood-water barrier of a fish and the SPMD membrane is similar.

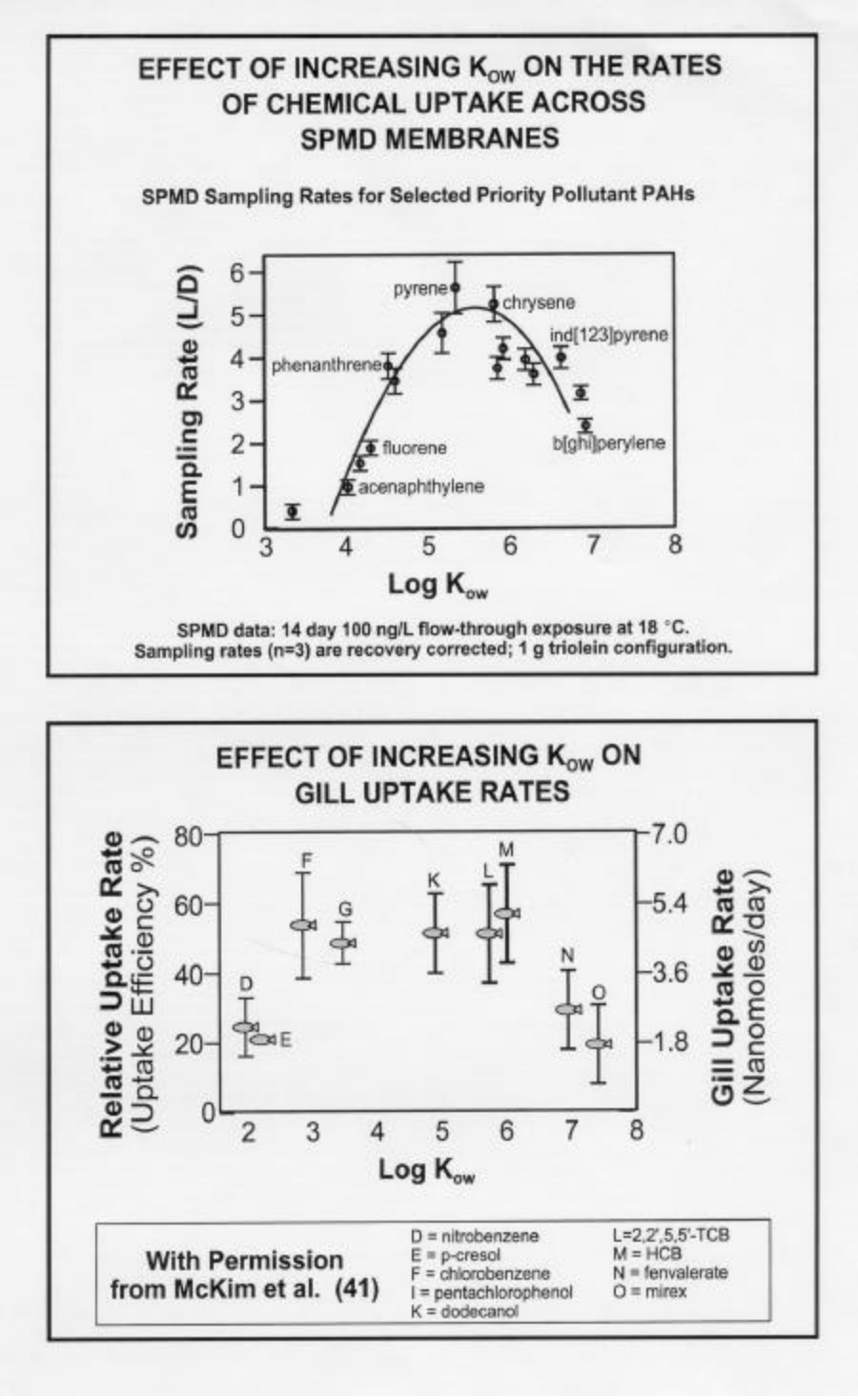

Figure 7.1. Comparison of the Patterns of Organic Contaminant Uptake Rates (as Related to Log K_{ow}s) by SPMD and Across Fish Gills (McKim *et al.*, 1985).

Table 7-1 (page 7-5) provides a comparison of the physical characteristics of the standard SPMD membrane and the respiratory lamellae (gills) of fish. Not shown in this comparison is the thickness of the associated aqueous boundary layers, which varies with flow velocity-turbulence. Except under very turbulent conditions, the effective thickness and related resistance, of the SPMD aqueous boundary layer is expected to be greater than that associated with the gills of an organism. This is due to the active pumping action that organisms use for respiration, and in some cases, feeding. In theory, the much higher surface area of the SPMD membrane should offset the greater uptake flux (based on mass or moles/d cm^2) of residues due to the probable lower resistance across the aqueous boundary layer of fish gills. Equation 7-1 shows the importance of exchanging membrane surface area and the associated boundary layer thickness in the relative rate of chemical uptake flux (F_c).

$$F_c = D_w A C_w / \quad l \qquad \text{(Equation 7-1)}$$

Clearly, the strength of the correlations between the rates of accumulation of chemicals by SPMDs and biomonitoring organisms is dependent on species type (e.g., rates of xenobiotic metabolism, diet, etc.), test conditions (includes organism stressors such as ammonia/ pH, food supply, etc., and environmental conditions such as flow velocity, temperature, etc.), and the types of chemicals examined. For example, data compiled by MacKay *et al*., 1992a showed that there are very large differences (i.e., up to $\approx 10^4$ fold) in PP PAH uptake rates (k_us) for different PAH structures and species of aquatic organisms. Just for anthracene, the k_us of test organisms vary by 2.5×10^2 fold. Unless extreme environmental conditions are included, SPMD k_us for an individual PP PAH should vary less than an order of magnitude.

Table 7-1. Comparison of the SPMD Membrane and Respiratory Lamellae (gills) of Fish.

Characteristic	Standard SPMD [a]	Fish [a]
Membrane:		
Composition	Low density polyethylene (LPDE)	Complex lipoprotein bilayer
Surface area	80 cm^2 / g SPMD	1 – 9 cm^2 / g tissue
Thickness	86 µm	0.5 – 11 µm Total distance of blood-water barrier
Uptake (Chemical) Rate Control:	Membrane if $K_{OW} < 4.5 \times 10^4$ Diffusion layer if $K_{OW} > 4.5 \times 10^4$	Membrane if $K_{OW} < 10^3$ Diffusion layer if $K_{OW} > 10^3$

[a] Data obtained from Hayton and Barron (1990); and Gobas *et al. (*1986).

In view of the large variability in literature uptake rate constants for organisms, it is unreasonable to expect SPMDs to mimic the accumulation of contaminants by all aquatic test species and under all test conditions. However, examination of the results of several side-by-side studies shows that the accumulation rates of residues by SPMDs and several biomonitoring organisms are often surprisingly close together. For example, Prest *et al.* (1992) found that the levels of PCDD/Fs were about 1.6 times higher in exposed clams (*Corbicula fluminea*, wet weight basis) than in similarly exposed SPMDs (lipid analyzed separately, residues in the membrane were not analyzed). Sabaliunas *et al.* (1998) showed that whole SPMD uptake rates of selected pesticides (i.e., chlordane, endosulfan, allethrin and fenvalerate) were 3.5 to 5.5 fold higher than the uptake rates of mussels (*Anodonta piscinalis*, wet weight). Also, the mussels appeared to reach steady-state concentrations in less than 20 d, whereas SPMD uptake appeared linear throughout the

20 d exposure. Meadows *et al.* (1998) found that the uptake rates of PCBs by brown trout (*Salmo trutta*, wet weight) and SPMDs (whole SPMDs) were similar. Both matrices exhibited linear uptake throughout the 28 d exposure. Uptake rates for SPMDs averaged about 2 fold greater than trout over a 500-fold range in PCB congener K_{ow} values and the correlation coefficient (R^2) for the uptake rate comparison was 0.89. Prest *et al.* (1997), showed that the laboratory uptake rates of 26 PAHs (log K_{ow}s ranging from about 4.0 to 6.9) by oysters (*Crassostrea gigas*, wet weight) and whole SPMDs were similar. The mean (n = 26) of the PAH uptake ratios obtained by dividing SPMD k_us by oyster k_us for the 26 PAHs was 1.4. On an organism dry weight basis (see comments on the appropriate data format for comparisons in "DATA FORMAT AND COMPARABILITY", page 5-14), the mean of the ratios is about 0.2. Uptake of the 26 PAHs by SPMDs remained linear throughout the twenty-day exposure, whereas oyster uptake was curvilinear (i.e., steady state is approached).

Table 7-2 (page 7-7) shows a comparison of whole SPMD and oyster (wet weight) exchange kinetics for selected PAHs (derived from data reported by Prest *et al*. 1997). Clearly, in this side-by-side study PAH k_us for SPMDs and oysters match fairly closely. This is not the case for the first-order half-lives ($t_{1/2}$s) of PAHs, as oyster $t_{1/2}$s were 15 to 25-fold less than SPMDs. Even without the potential for metabolically mediated depuration of chemicals by bivalves (see work by Prest *et al*., 1995b, given in "EXAMPLES OF APPLICATIONS" page B-1 for an example of possible metabolism of contaminant residues), other reasons exist for longer half-lives of contaminants in SPMDs. Prest *et al.* (1992, 1995a, 1997) has suggested that a significant portion of high K_{ow} residues measured in filter-feeding bivalves remain in the gut contents and are not assimilated into tissues. The excretion of this fraction of chemicals is much more rapid than the diffusion-limited clearance of residues incorporated in tissues. Also, on a unit mass basis, the lipid content of SPMDs is generally more than 10-times greater than shellfish and more than 5-times greater than finfish. Also, unlike non-lipoidal

Table 7-2. Comparison of SPMD and Oyster (*Crassotrea gigas*) Exchange Kinetics for Selected PAHs.

	SPMDs			*Oysters*			
Compounds	k_u ($Lg^{-1}d^{-1}$)	k_e (t^{-1})	$t_{½}$ (d)	k_u (wet)	k_u (dry)	k_e (dry)	$t_{½}$ (d)
1-ethylnaphthalene	0.49	0.021	33	0.30	1.84	0.314	2.2
1,3-dimethylnaphthalene	0.50	0.011	63	0.33	2.07	0.281	2.5
fluorene	0.46	0.019	36	0.30	1.85	0.314	2.2

tissues, the SPMD membrane adds significantly to the overall capacity of SPMDs (i.e., the K_{SPMD}) for contaminants. The following equations show the effect of lipid content, the SPMD membrane, and non-lipoidal tissues on the magnitude of the clearance rate constants (k_es) for SPMDs and biomonitoring organisms.

$$k_{e\text{-}SPMD} = -k_o K_{mw} A / K_{Lw} V_L + K_{mw} V_m \qquad \text{(Equation 7-2)}$$

$$k_{e\text{-}BM} = -k_o K_{mw} A / K_{Lw} V_L + K_{nw} V_t \qquad \text{(Equation 7-3)}$$

The subscript "$_{BM}$" and "n" in Equation 7-3 represents biomonitoring organisms and non-lipoidal tissues, respectively. Obviously, an increase in V_L will lower the magnitude of both $k_{e\text{-}SPMD}$ and $k_{e\text{-}BM}$. Thus, it appears that the low lipid content (V_L), coupled with low values for K_{nw}, rapid depuration of gut contents, and potential biotransformation and active excretion of metabolites mediate the observed lower $t_{1/2}$s of chemicals in organisms relative to SPMDs. Figure 7-2 illustrates this difference in $t_{1/2}$s or residence times for alkylated naphthalene in SPMDs and oysters, and clearly shows that SPMDs are much better suited to detect episodic releases of chemicals. Note that in the case of PAHs, the ability of biomonitoring organisms to rapidly depurate accumulated residues is as follows: mammals > fishes > crustaceans > bivalve mollusks (Huckins *et al.,* 1997c).

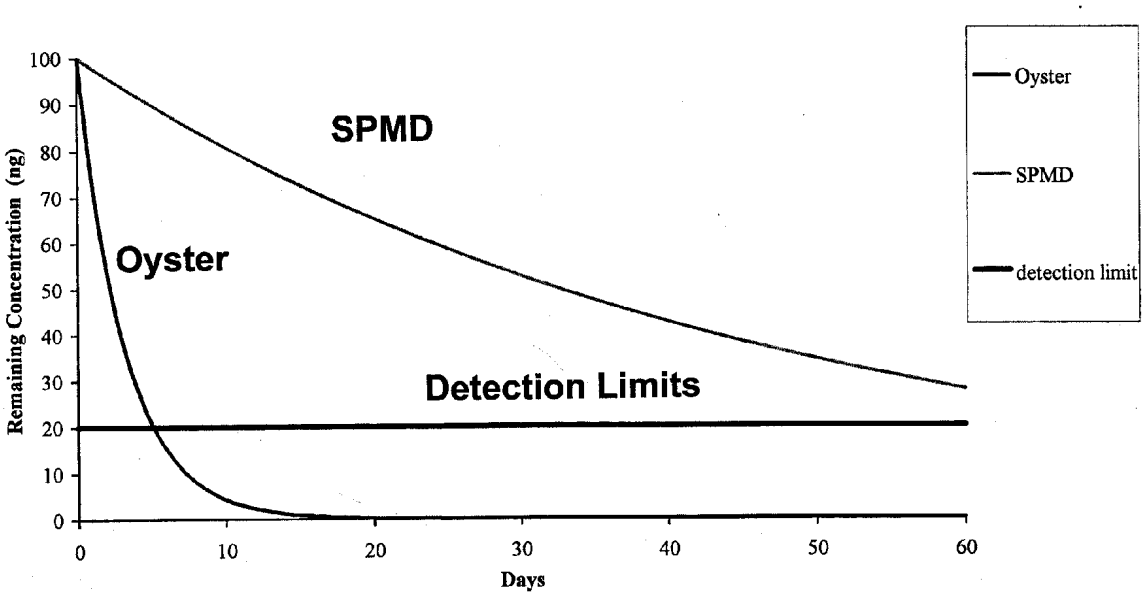

Figure 7-2. Comparison of alkylated naphthalene residence times in SPMDs and oysters (*Crassotrea gigas*). *Note that bivalves, such as oysters are known to have very low or no detectable concentrations of mixed function oxygenase enzymes.*

Obviously, the use of SPMDs to monitor hydrophobic contaminant levels and to estimate organism exposure has a number of advantages over the use of biomonitoring organisms. Some of these include: 1) the materials used to construct SPMDs are reproducible and can be obtained with very low background levels of contaminants, while it is often difficult to obtain test organisms with low levels of target contaminants, 2) SPMDs accumulate a broader range of hydrophobic organic compounds (See Figures B-1, page B-4 and B-2, page B-5), especially moderate to low K_{ow} compounds, 3) unlike biomonitoring organisms, SPMDs are not affected by most water quality parameters (e.g., salinity, ammonia and anthropogenic contaminant levels) and transplant issues (e.g., the geographical range of available test organisms, lack of food at test site and other caging effects, dramatic changes in flow regimes) 4) obviously, differences in anatomical, physiological and behavior of biomonitors are not relevant to the accumulation of contaminant residues in passive samplers, 5) because SPMDs have higher lipid contents than nearly all organisms, and only passively dissipate accumulated chemicals, the $t_{1/2}$ or residence time of residues

and the integrative (linear) phase of analyte uptake (see Figure 2-1, page 2-6) are expected to be substantially greater than for biomonitors, and 6) dissolved residues accumulated by SPMD are clearly bioavailable, whereas the possible contribution of non-incorporated residues (tissues) in the gut complicates the estimation of contaminant bioavailability from chemical body burdens in whole organisms.

To avoid analysis of non-tissue incorporated residues, biomonitoring organisms are often allowed to depurate gut contents prior to tissue analysis. However, based on the relatively large clearance rate constants (k_es) of many contaminants (Mackay *et al*., 1992a), depuration of gut contents will inevitably lead to some losses of tissue associated residues with high to moderate fugacity. For example, Moring and Rose (1997) have suggested that the depuration of *Corbicula fluminea* prior to analysis was a possible factor in the low numbers and levels of PP PAHs detected in tissues, relative to co-deployed SPMDs (see "EXAMPLES OF APPLICATIONS", page B-1).

The comments presented thus far are not intended to imply that biomonitoring organisms are not useful for addressing environmental contaminant issues. Obviously, biomonitoring organisms are needed to confirm the direct toxicity or more subtle adverse effects of contaminants in environmental waters. Also, some biomonitoring organisms appear to sample very high K_{OW} compounds at greater rates than SPMDs. Based on bioaccumulation literature (Connell, 1990) the dietary route of uptake becomes dominant when a compound's log K_{ow} exceeds about 6.5. Finally, Herve *et al*. (1995) showed that mussels sampled chlorophenols with three or more chlorines, while they were not detected in side-by-side deployed SPMDs. On the other hand, SPMDs sampled chlorophenols with two chlorines and polychlorinated -anisoles and -veratroles, which were not detected in the mussels. The inability of SPMDs to sample more chlorinated phenols is due to the low pK_a (i.e., ionization constant) of these compounds. Because the pH of water at the study site (Herve *et al*., 1995) was one or more log units greater than the pK_as of chlorophenols with three or more chlorines, their water borne residues will be largely ionized. Clearly, SPMDs

do not sample ionic species whereas bivalves do. Thus, for some environmental contamination scenarios, the two approaches are complementary.

Several investigators have noted that SPMDs often contain higher concentrations of low K_{ow} compounds than biomonitoring organisms (Prest *et al*., 1992; Prest *et al.,* 1995b; Peven *et al*., 1996). A number of reasons exist for this apparent disparity. These include the following: 1) SPMD concentrations are proportional to dissolved water concentrations, which is often not the case for aquatic organisms, 2) because water solubility is inversely proportional to compound K_{ow}, higher concentrations of low K_{ow} compounds are present in waters from many environmental release scenarios, 3) the relatively low lipid content of organisms results in the rapid saturation of compounds with relatively low and moderate K_{ow}s (i.e., concentrations don't rise during the rest of the exposure), while concentrations of compounds with high K_{ow}s generally rise throughout the exposure period (also note that often the concentrations of compounds with relatively low and moderate K_{ow}s usually rise in SPMDs at about the same rate throughout an exposure period), and 4) biotransformation/metabolism appears to be more prevalent for compounds with lower K_{ow}s.

Gale *et al*. (1997), Meadows *et al*. (1998), Prest *et al*. (1997), and others have shown, in side-by-side exposures of SPMDs and biomonitoring organisms to complex contaminant mixtures, that the profiles or fingerprints of accumulated residues in the two matrices are similar. In general, it appears that the major difference between studies that show similarities and dissimilarities between accumulated residues in SPMD and biota is the types of compounds targeted and the role of dietary uptake. If the target compounds are environmentally persistent (i.e., not readily biotransformed), have high K_{ow}s, and dietary uptake is limited, the similarities between the two sampling matrices are maximized. Finally, differences are expected to be the greatest between residue patterns in SPMDs and biomonitoring organisms when feral organisms are used, because of factors such as mobility or adaptability.

Section 8

CONCLUSIONS

For about a decade, SPMDs have been used in diverse environmental contaminant studies. Most of these investigations can be categorized as follows: 1) contaminant detection, sources, transport-fate, and exposure to organisms, 2) comparisons or evaluations of SPMDs and organisms for monitoring contaminants, 3) studies of SPMDs as a mimetic (water) preconcentration method for toxicity screening (e.g., biomarker/bioassay and immunoassay tests), and 4) use of the SPMD dialysis method for enrichment of environmental samples.

In this guide, we describe the use and utility of SPMD technology for nearly all of these applications. Theory is extended on mechanisms governing the uptake and elimination of hydrophobic organic contaminants by whole SPMDs (lipid and membrane), and mathematical models are presented that permit correlation of residue concentrations in whole SPMDs to ambient TWA levels of the same chemicals. The potential effects of exposure conditions on SPMD sampling rates and the resulting errors in water concentration estimates are explained. Also, theory and experimental evidence is presented that suggests errors in water concentration estimates can be minimized by using PRCs or by selecting or controlling exposure conditions to more closely match calibration conditions. Procedures are delineated for the preparation, transport, and deployment of SPMDs that maintain sample integrity by minimizing the potential for analytical interferences (artifacts in some cases) and by ensuring the preservation of residues accumulated in SPMDs. Methods are also presented for the enrichment/cleanup of SPMD samples and the degree of sample cleanup needed is shown to depend on the nature of the application and the sampling site. For example, solutions of non-enriched lipid and membrane extracts have been successfully used for Microtox and Mutatox toxicity

screens, whereas analytical determinations of PAH concentrations in SPMDs requires almost as many enrichment steps as the analysis of PAHs in bivalves. We provide general guidelines and give examples of the assay of SPMD extracts with selected biomarkers or bioassays. The use of SPMDs with biomarkers is complementary because biomarker assays are generally not sensitive enough to directly measure the toxicity of trace-waterborne residues and do not account for contaminant bioconcentration. Even in studies where exposure assessment is the primary goal (i.e., toxicity is not considered), biomarker screening can sometimes be employed to focus costly analytical efforts. Also, reasons for observed similarities and dissimilarities between the accumulation of contaminants by SPMDs and biomonitoring organisms are given. Often the two monitoring approaches are complementary, as each method can provide different kinds of information.

Unlike all other approaches (analytical [e.g., LLE and SPE] and biomonitoring), SPMDs are shown to be selective for the dissolved phase of aquatic residues. SPMDs are the only demonstrated *in situ* passive monitoring approach for sampling sub-part-per-quadrillion levels of dissolved residues such as dioxins (Lebo *et al*., 1995; Gale *et al*., 1997; McCarthy and Gale, 1999). The dissolved fraction is very relevant to environmental risk assessment, because it is clearly bioavailable and most aquatic toxicity data are based on dissolved phase concentrations. To our knowledge, SPMDs have not been used in projects, where litigation was involved. However, few technical barriers exist to the validation and general acceptance of SPMDs for monitoring aquatic environments. In general, SPMD data appears to be on par with the most accurate screening methods.

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Appendix A

SOURCES OF SPMD INFORMATION

Since the inception of SPMDs in the late nineteen eighties (Huckins, 1989), research on the devices has grown rapidly. The results of this work has been reported in the form of abstracts from presentations, laboratory reports and letters to journal editors, a "learned discourse" for the Society of Environmental Toxicology and Chemistry News, peer-reviewed journal articles, book chapters, PhD and masters theses, and electronic media. Herein, we provide the following current list of SPMD-related peer-reviewed articles alphabetized according to lead author. Also included are book chapters, letters to editors, selected theses, and Internet addresses for SPMD information.

JOURNAL ARTICLES AND BOOK CHAPTERS

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Discussion Group for Users of SPMD Technology: <SPMD_sampling@listserv.umu.s

Appendix B

EXAMPLES OF APPLICATIONS

In this work we have shown that SPMDs are suitable for use in both environmental exposure and toxicity assessments. More specifically, documented applications (aquatic environments) of SPMD technology include: 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 chemical exposure. Because some of these applications are interrelated, most SPMD studies address more than one of the listings above.

Herein, we provide several examples of field studies that cover most of the applications listed above. Lebo *et al*. (1992) have described the details of an urban freshwater deployment designed to detect suspect PAH contamination. Briefly, the SPMDs were deployed for 21 days at several locations in a small Mid-western creek (Flat Branch Creek, Columbia, MO). After recovery of the sampler and cleanup of SPMD extracts, gas chromatographic analysis of the purified sample revealed the presence of a point source input of petrogenic (petroleum derived) PAHs. The reference site (above the input source) contained minor amounts of PAHs typical of pyrogenic (combustion derived) residues. Samples from the site adjacent (just downstream) to the suspect source were determined to contain about 0.5 mg total aromatics per 1.0-g triolein SPMD and sample residue profiles were dominated with PAHs characteristic of a petrogenic source. Samples from SPMDs deployed farther downstream from the input source contained decreasing levels of PAH residues, with the most downstream site (about 1 Km) containing essentially background levels. This study illustrated the utility of SPMD to determine the presence and sources of contamination (i.e., application 1).

On a much larger scale, McCarthy and Gale (1999) used SPMDs to determine the presence, sources and dynamics of hydrophobic organic contaminants in the lower Columbia River Basin. This application of SPMD technology permitted the detection of the presence of target analytes, such as PCDDs and PCDFs, at environmental concentrations as low as < 1 fg/L, or less than part-per-quintillion levels. Sources of elevated contaminant concentrations were readily located, and the dilution effects of high-flow conditions were delineated. The latter finding directly relates to the determination of chemical transport and fate listed in the above applications. This study also demonstrated that, unlike most conventional sampling approaches, SPMDs could reveal patterns of the distribution of extremely toxic compounds at environmentally relevant concentrations. In another large-scale study, Bergqvist *et al*. (1998b) used SPMDs to monitor the transport/fate of contaminants (along the Swedish west coast) in a large floodwater plume from Central and Western Europe. By replacing deployed SPMDs through time, the arrival of the floodwater plume was detected and linked to elevated concentrations of pollutants, followed by declining concentrations through time. Clearly, this study also demonstrates that SPMDs are powerful tools for monitoring the transport/fate of waterborne chemicals.

In all the studies cited above, TWA concentrations of contaminants were derived from the levels measured in SPMDs. Thus, application 2, as described above, is generally an integral part of most SPMD related contaminant assessments.

Because SPMDs integratively sample most dissolved high K_{ow} compounds, the time weighted flux of these residues (i.e., mass per unit time) can often be estimated for a drainage system. McCarthy, *et al*. (2000) demonstrated the use of SPMDs for estimating the contribution of three US Department of Energy (DOE) industrial and research facilities to the regional PCB budgets. The flux of PCBs from these facilities was calculated from TWA PCB concentrations in effluents (derived from SPMD exposures) and estimates of the volumetric flow rates of discharges and receiving streams during the deployment period. Using this approach, McCarthy *et al*. (2000) found that PCBs from these DOE

facilities constituted only about 10% of the total PCB load in the Clinch River. Also, principle component analysis of the concentrations of PCB congeners in SPMDs facilitated determination of specific PCB sources. This work encompassed aspects of applications 1 and 2 listed above, but is one of the only examples of application 3.

Bivalves are widely used in monitoring programs to assess the waterborne contaminant exposure (see SPMD application 5 above). These organisms are used because they are immobile, widely available, lack significant levels of certain enzyme systems known to mediate the metabolism of many contaminants, and ventilate large volumes of water (see "COMPARISONS TO BIOMONITORING ORGANISMS" for additional details). However, several investigators have observed the lack of proportionality between contaminant exposure levels and residue body burdens in bivalves. The following examples illustrate this potential problem. Prest *et al*. (1995b) deployed mussels (*Mytilus edulis*) and SPMDs contiguously at several sites, including sites near a refinery effluent, in Corio Bay, Victoria, Australia, to examine their relative abilities to monitor a known gradient of chlorinated contaminants. Overall, the levels of chlorinated organic chemicals were about the same in both sample types, however, the GC chromatograms from the two matrices differed markedly as shown in Figure B-1, page B-4. Data from analysis of the SPMD samples suggested that lower chlorinated PCBs and a complex mixture of unknowns (early eluting components) were present at high levels in the water column, while data from the mussel samples implied essentially the reverse. These results are not surprising for several reasons. Unlike aquatic organisms, concentrations of hydrophobic organic contaminants in SPMDs have been shown to be proportional to ambient water concentrations. Also, early eluting (GC) chlorinated organics are more soluble in water and would be expected to be present at higher concentrations in aquatic environments than higher molecular weight (later eluting) components. With respect to aquatic organisms, an inverse relationship has been shown to exist between log K_{ow} and depuration rate constants (k_es) of OCs (Fox *et al*., 1994; Prest *et al.,* 1997).

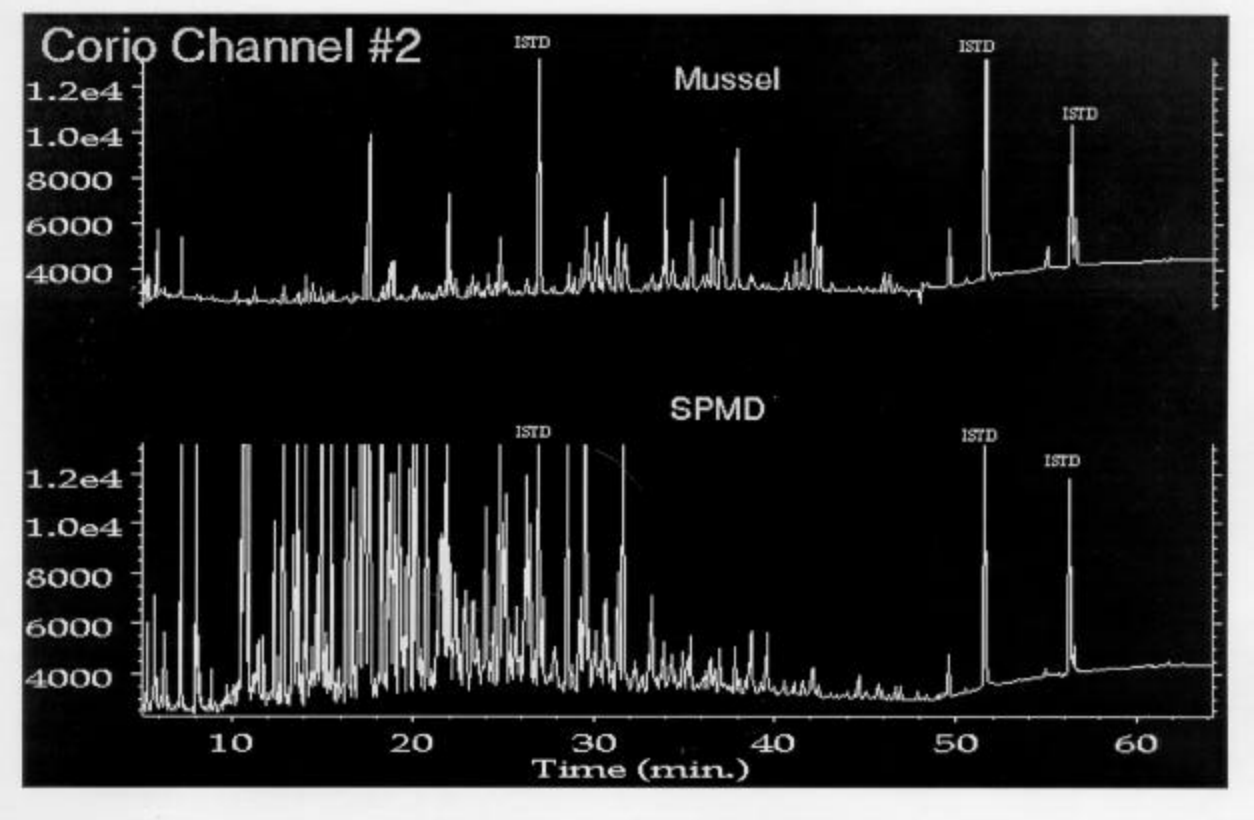

Figure B-1. GC-MS comparison of ion chromatograms of extracts from mussels (*Mytilus edulis*) and SPMDs, Corio Bay Australia.

One of the SPMD deployment sites was in a refinery effluent stream where bivalves could not survive due to elevated temperature and turbidity. This study demonstrates that unlike aquatic organisms, SPMD concentrations appear to be proportional to the waterborne concentrations of a much wider range of chemicals.

SPMDs and Asiatic clams, *Corbicula fluminea* were deployed at stream sites in the Dallas-Fort Worth Metropolitan Area (Moring and Rose, 1997) to assess the presence and concentrations of bioavailable, dissolved PAHs. Twenty-four PAHs were detected

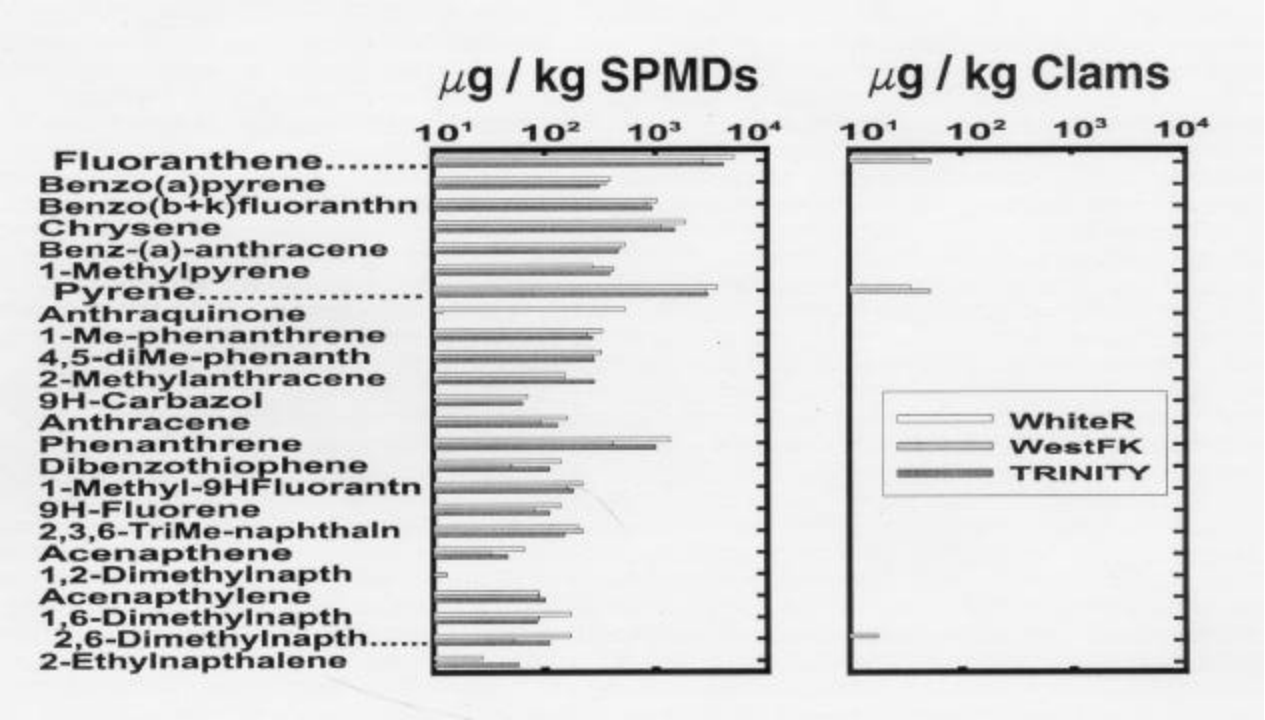

Figure B-2. Comparison of PAHs detected by GC-PID in SPMDs and clams (*Corbicula fuminea*), Trinity River, Dallas, Texas (Moring and Rose, 1997).

in SPMDs, 20 of which occurred at all sites and only three PAHs were detected in the co-deployed clams (Figure B-2). Throughout all sites, non-alkylated PAHs were found at greater levels in SPMDs than alkylated forms. Nine of 16 Priority Pollutant PAHs were detected in SPMDs. In several cases (i.e., benz (a) anthracene, benzo (a) pyrene, and chrysene), estimated concentrations in water exceeded the U.S. Environmental Protection Agency's human health criteria. This example of SPMD field applications illustrates that exposure to potentially toxicologically significant residues of PAHs in surface waters may not be detected when using a biomonitoring organism. A possible explanation for this shortcoming is the presence of stressors, other than the contaminants of concern, affecting bivalve feeding and respiration.

Under more optimal exposure conditions (e.g., absence of environmental stressor such as extremes in water quality and caging effects) for biomonitoring organisms and for

persistent compounds that are known to bioaccumulate, the uptake of chemicals by SPMDs and aquatic organisms can be remarkably similar. For example, Prest *et al*. (1997), Huckins *et al*. (1998b), and Meadows *et al*. (1998) have shown that the uptake rates (k_us) of PAHs and PCBs by SPMDs (standard whole SPMD) and biomonitoring organisms are similar in magnitude, i.e., < 2 fold difference (see "Comparison to Biomonitoring Organisms", page 7-1). More recently, Echols *et al*. (2000), found less than a three-fold difference in the concentrations of PCBs accumulated by caged channel catfish and standard whole SPMDs. These studies illustrate that SPMDs concentrate hydrophobic organic pollutants mimetically for use in application 4 above, and often provide an improved indicator of aquatic organism exposure to water borne contaminant residues (i.e., the justification for application 5).

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Appendix C

ADDITIONAL CALIBRATION DATA

The following tables are from several authors and provide calibration data for PCBs, PCDDs, PCDFs and selected insecticides.

Table C-1. PCB Congener Uptake Rate Constants for SPMDs. [a,b]

(Congeners) IUPAC No.	Log K_{ow}	R_s (L/d)	S.D.	k_u (mL/d·g)	S.D.
006	5.1	12.8	3.5	2,900	800
018	5.2	9.2	2.2	2,100	500
019	5.0	5.3	0.9	1,200	200
022	5.6	5.7	0.9	1,300	200
025	5.7	5.7	0.9	1,300	200
026	5.7	5.7	0.9	1,300	200
028	5.7	8.4	1.8	1,900	400
031	5.7	7.0	3.1	1,600	700
040	5.7	6.6	1.8	1,500	400
041	5.7	6.2	2.2	1,400	500
042	5.8	6.2	1.3	1,400	300
043	5.8	6.2	0.9	1,400	200
044	5.8	7.5	1.8	1,700	400
045	5.5	7.9	1.8	1,800	400
046	5.5	4.4	0.4	1,000	100
047	5.8	7.5	2.2	1,700	500
048	5.8	3.5	0.0	800	0
049	5.8	5.3	0.9	1.200	200
051	5.6	4.8	0.9	1.100	900
052	5.8	6.2	0.9	1.400	200
053	5.6	4.8	0.9	1.100	200
063	6.2	5.3	2.2	1.200	200
064	6.0	7.5	2.2	1.700	500
066	6.2	5.3	1.3	1.200	300

Table C-1. Continued.

(Congeners) IUPAC No.	Log K_{ow}	R_s (L/d)	S.D.	k_u (mL/d/g)	S.D.
067	6.2	5.3	0.9	1,200	200
070	6.2	7.0	1.8	1,600	400
074	6.2	6.2	2.6	1,400	600
081	6.4	4.8	1.3	1,100	300
082	6.2	4.4	0.4	1,000	100
083	6.3	4.8	0.4	1,100	100
084	6.0	4.4	0.9	1,000	200
085	6.3	4.8	0.9	1,100	200
087	6.3	5.3	1.3	1,200	300
090	6.4	6.2	0.9	1,400	200
091	6.1	4.4	0.4	1,000	100
092	6.4	5.3	0.9	1,200	200
095	6.1	6.2	1.8	1,400	400
097	6.3	4.4	0.4	1,000	100
099	6.4	4.4	0.9	1,000	100
101	6.4	6.2	1.3	1,400	300
105	6.6	4.0	1.3	900	300
107	6.7	5.3	0.9	1,200	200
110	6.5	5.7	2.2	1,300	500
114	6.6	4.4	0.9	1,000	200
118	6.7	4.8	0.4	1,100	100
119	6.6	4.4	0.4	1,000	100
128	6.7	4.4	0.9	1,000	200
129	6.7	3.5	0.4	800	100

Table C-1. Continued.

(Congeners) IUPAC No.	Log K_{ow}	R_s (L/d)	S.D.	k_u (mL/d²g)	S.D.
130	6.8	4.0	0.9	900	200
134	6.6	4.8	0.4	1,100	100
136	6.2	5.3	1.3	1,200	300
137	6.8	3.5	0.9	800	200
138	6.8	4.8	0.4	1,100	100
141	6.8	4.8	1.3	1,100	300
146	6.9	4.8	2.2	1,100	200
149	6.7	5.7	0.4	1,300	100
151	6.6	5.3	0.9	1,200	200
153	6.9	3.2	0.9	600	100
156	7.2	2.6	0.9	600	200
157	7.2	2.6	0.4	600	100
158	7.0	3.5	0.9	800	200
172	7.3	1.3	0.0	300	0
174	7.1	3.1	0.9	700	200
176	6.8	2.2	0.9	500	200
178	7.1	3.1	0.4	700	100
179	6.7	2.2	0.4	500	100
180	7.4	2.6	0.4	600	100
183	7.2	3.1	0.4	700	100
187	7.2	3.5	0.4	800	100
194	7.8	1.3	0.4	300	100
199	7.6	1.8	0.4	400	100
201	7.3	1.8	0.4	400	100

Table C-1. Continued.

(Congeners) IUPAC No.	Log K_{ow}	R_s (L/d)	S.D.	k_u (mL/d/g)	S.D.
207	7.7	0.4	0.0	100	0
3PCBs [c]	6.4	4.8	--- [d]	1,100	---

[a] Data from Meadows *et al*. (1998) are based on standard whole SPMD.
[b] Exposure duration was 28 days, water temperature 11.8 ^{o}C and flow velocity was < 1cm/sec.
[c] Values for total PCBs (SPCBs) are averages of individual congener values.
[d] Not calculated.

Table C-2. Whole SPMD (lipid plus membrane) Uptake Rates (11 °C and 19 °C) for PCDDs, PCDFs and PCBs [a,b]

Compounds	11 °C		19 °C	
	R_s (L/d)	k_u (mL/d g)	R_s (L/d)	k_u (mL/d g)
2,3,7,8-TCDD	2.5	790	3.8	1,190
1,2,3,7,8-PeCDD	1.8	570	3.4	1,070
1,2,3,4,7,8-HxCDD	1.4	440	4.0	1,260
1,2,3,6,7,8-HxCDD	1.4	440	3.2	1,010
1,2,3,7,8,9-HxCDD	1.3	410	2.9	910
1,2,3,4,6,7,8-HpCDD	0.7	220	2.2	690
OCDD	1.3	410	3.0	940
2,3,7,8-TCDF	2.5	790	3.7	1,160
1,2,3,7,8-PeCDF	2.0	630	3.8	1,190
2,3,4,7,8-PeCDF	1.9	600	4.2	1,320
1,2,3,4,7,8-HxCDF	1.2	380	2.7	850
1,2,3,6,7,8-HxCDF	1.2	380	2.9	910
2,3,4,6,7,8-HxCDF	1.2	380	3.0	940
1,2,3,7,8,9-HxCDF	1.0	310	2.3	720
1,2,3,4,6,7,8-HpCDF	0.7	220	2.7	850
OCDF	0.5	160	1.8	570
PCB-77	2.9	910	4.4	1,380
PCB-78	4.4	1,380	5.2	1,640
PCB-79	5.1	1,600	5.2	1,640
PCB-81	3.7	1,160	4.9	1,540

Table C-2. Continued

Compounds	11 °C		19 °C	
	R_s (L/d)	k_u (mL/d g)	R_s (L/d)	k_u (mL/d g)
PCB-126	2.2	690	4.2	1,320
PCB-127	1.6	500	4.1	1,290
PCB-169	2.1	660	5.9	1,860

[a] Data from Rantalainen *et al*. (2000); DD, dibenzodioxins, DF, dibenzofurans, and numbers following PCB are IUPAC numbers for congeners.

[b] Water Flow rate < 1 cm/sec.

Table C-3. Physicochemical Properties and Standard SPMD (Whole Device) Uptake Kinetic Parameters (10 °C) for Selected Insecticides [a]

Compounds	MW	Log K_{ow}	k_u (mL/d.g)	CV (%)	R_s [b] (mL/d)
Endosulfan	407	5.4	201	4.3	1,108
Allethrin	302	5.0	58	4.6	322
Fenvalerate	420	4.42 - 6.2	77	6.9	422

[a] Data from Sabaliunas *et al*. (1998), exposure period 20 days.
[b] 1-g Triolein SPMD.

Appendix D

WATER CONCENTRATION EXTRAPOLATION

The models used to relate SPMD concentrations to ambient water concentrations are given in Section 3, “THEORY AND MODELING”. However, selection and parameterization of the appropriate models is dependent on a number of factors, which include exposure period and environmental conditions, properties of target analytes, and the availability of data from laboratory calibration studies and PRCs. Assuming analyte concentrations (includes PRC levels when applicable) in exposed SPMDs have been measured, the first step in the water-concentration derivation process is to compare site exposure conditions (i.e., temperature, hydrodynamics, and level of biofouling) to those used to generate SPMD calibration data. When significant differences do exist, then adjustments to SPMD sampling rates (i.e., $k_{u\text{-}cal}$s or $R_{s\text{-}cal}$s), derived from controlled calibration studies, must be considered. If PRCs are used in exposed and field blank SPMDs, sampling rate adjustments are relatively straight forward, as shown in Equations 3-31 and 3-32. Note that $k_{e\text{-}PRC}$s are determined by Equations 3-33 or 3-34 and that values for analyte K_{SPMD}s are needed (see discussion on K_{SPMD}s below). In cases where PRCs are not used, adjustments to SPMD $k_{u\text{-}cal}$s or $R_{s\text{-}cal}$s can be made based on reports on the effects of environmental variables given in this or other documents, but the justification for changing calibration data is less certain.

After deriving or obtaining R_ss or k_us appropriate for exposure sites, the next step in the water concentration derivation process is the determination of analyte K_{ow}s. If analyte K_{ow}s are not found in this document, a large number of K_{ow}s are available from several on-line databases (e.g., Syracuse Research Corporation) and other sources such as Mackay *et al.* (1992a), Mackay *et al.* (1992b) and Mackay *et al.* (1997). Also, a number of computational approaches exist to derive K_{ow}s (e.g., Leo’s fragment constant method, Lyman et al., 1982). When analyte log K_{ow}s are greater than 6.0, the use of Equation 3-11

(i.e., a linear uptake model) is generally recommended for estimation of water concentrations. Note that for extended exposures (i.e., > 30 d) in warm, high flow/turbulent waters, the use of the curvilinear model (Equation 3-10) may be necessary. In any case, the use of Equation 3-18 is recommended for assessing the kinetic phase (see Figure 2-1, page 2-6) of analyte uptake during the exposure of interest. Equation 3-18 requires knowledge of analyte K_{SPMD}s, but if screening is the goal, K_{ow}s can be substituted for K_{SPMD}s. Few K_{SPMD} values are available in the literature but K_{SPMD}s can be derived with Equations 3-2, 3-5, and 3-6, if the log K_{ow} is not greater than 6.0.

Following the rationale given in the "THEORY AND MODELING" section, page 3-1, a linear model (Equation 3-11) is used to calculate TWA water concentrations of analytes, when exposure time is less than the computed t_{50} (Equation 3-18). If exposure time (t) divided by t_{50} (t/t_{50}) is between 1.0 and 4.0 Equation 3-10 (curvilinear uptake) is recommended for computation of ambient water levels. Finally, when t/t_{50} is > 4.0, the use of Equation 3-14 (attainment or close approach of equilibrium) is appropriate for water concentration derivation.

To facilitate the water concentration estimation process, we have developed a Microsoft Excel® 2000 spreadsheet, which used the logic and models given above to estimate ambient water concentrations from SPMD levels of analytes of interest. The spreadsheet also allows the use of PRCs, as described above, for the derivation of site specific SPMD k_us and R_ss. This calculator only applies to lipid containing SPMDs of standard design (see "STUDY CONSIDERATIONS", page 4-1). The user only needs to enter data for variables such as temperature, exposure time, SPMD dimensions and measured values of PRC concentrations and analyte concentration in the whole SPMD.

The following shows three examples of the use of the spreadsheet and the steps involved in the calculations. Tables D-1 and D-2 are 21 day SPMD exposures at 10 °C and 18 °C. Table D-3 is a 60 day SPMD exposure at 26 °C. The appropriate exposure time and

water temperature must be entered into the spreadsheet in order to correctly estimate the water concentrations of the compounds.

USE OF A EXCEL® CALCULATOR FOR ESTIMATING WATER CONCENTRATIONS FROM SPMD DATA

For estimating water concentrations (C_w) from SPMD data, enter the appropriate information into the corresponding cells. Then, enter a temperature value (i.e., 10, 18, or 26 °C, or any temperature assuming calibration data are available or can be derived), which most closely approximates the actual exposure water temperature.

Temperature (°C) = 10 **Exposure Time (d) =** 21

Mass of SPMD (g) = 4.5 (NOTE: a standard 91.4 cm SPMD has a mass of ≈4.5g)

Volume of Lipid (L) = 0.001 **Volume of Membrane (L**) = 0.0037

Volume of SPMD (L) = 0.0047

(NOTE: a standard 91.4 cm SPMD has lipid volume of 0.001 L. membrane volume of 0.0037 L, and a total volume of 0.0047 L.)

If a PRC was used, the $k_{e\text{-}PRC}$ can be calculated by $k_{e\text{-}PRC} = [\ln (C_{SPMDo}/C_{SPMD})]/t$. If a PRC was not used, enter the same number for the $k_{e\text{-}PRC}$ as for the $k_{e\text{-}cal}$. The $k_{e\text{-}cal}$ value is the laboratory calibration value for the native PRC analog.

$k_{e\text{-}PRC}$ (d^{-1}) = 0.021

$k_{e\text{-}cal}$ (d^{-1}) = 0.021 (NOTE: the $k_{e\text{-}cal}$ for D_{10}-Phenanthrene is 0.021 d^{-1})

Currently, estimated water concentrations cannot be calculated for all compounds because of lack of calibration data. For compounds in which laboratory R_s values do not exist, the term N/A will appear in place of a numerical value, indicating the inability to estimate the water concentration. The final Estimated Water Concentration values appear in the Estimated Water Concentration cells. Note that data are generally corrected to two significan figures.

Table D-1. Estimate of Water Concentration from SPMDs Exposed to 10 °C Water for 21 Days Using the Excel® Calculator.

OC Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Hexachlorobenzene	5.71 [b]	1.45E+05	2.6	2.6	182.3	10.0	183.2	linear
Pentachloroanisole	5.48 [c,f]	1.05E+05	5.1	5.1	67.1	10.0	93.4	linear
α-BHC	3.86 [b]	5.06E+03	0.9	0.9	18.3	10.0	231.1	curvilinear
Lindane	3.71 [b]	3.57E+03	0.7	0.7	16.6	10.0	348.5	curvilinear
β-BHC	3.86 [b]	5.06E+03	N/A	N/A	N/A	10.0	N/A	linear
Heptachlor	5.19 [b]	6.72E+04	3.6	3.6	60.8	10.0	132.3	linear
d-BHC	4.12 [b]	9.01E+03	N/A	N/A	N/A	10.0	N/A	linear
Dacthal	4.26 [b]	1.21E+04	0.6	0.6	65.8	10.0	793.7	linear
Oxychlordane	5.48 [b]	1.05E+05	2.9	2.9	118.0	10.0	164.2	linear
Heptachlor Epoxide	4.51 [b]	2.00E+04	2.9	2.9	22.5	10.0	164.2	linear
trans-Chlordane	5.38 [b,d,e,f]	9.05E+04	3.5	3.5	84.2	10.0	136.1	linear
trans-Nonachlor	6.35 [d,f]	3.16E+05	3.6	3.6	286.1	10.0	132.3	linear
o,p'-DDE	5.56 [b]	1.18E+05	3.3	3.3	116.5	10.0	144.3	linear
cis-Chlordane	5.38 [b,d,e,f]	9.05E+04	3.8	3.8	77.6	10.0	125.3	linear

Table D-1. Continued.

OC Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Endosulfan	3.78 [b]	4.21E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-DDE	6.14 [b]	2.50E+05	5.5	5.5	148.2	10.0	86.6	linear
Dieldrin	4.60 [b]	2.38E+04	1.8	1.8	43.1	10.0	264.6	linear
o,p'-DDD	6.08 [b]	2.33E+05	3.3	3.3	230.1	10.0	144.3	linear
Endrin	4.63 [b]	2.52E+04	3.1	3.1	26.5	10.0	153.6	linear
cis-Nonachlor	6.20 [d,f]	2.68E+05	2.8	2.8	311.8	10.0	170.1	linear
o,p'-DDT	5.59 [b]	1.23E+05	2.2	2.2	182.3	10.0	216.5	linear
p,p'-DDD	5.75 [b]	1.54E+05	3.1	3.1	161.3	10.0	153.6	linear
Endosulfan-II	3.50 [f]	2.15E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-DDT	5.47 [b]	1.04E+05	3.2	3.2	105.4	10.0	148.8	linear
Endosulfan Sulfate	3.64 [f]	3.02E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-Methoxychlor	4.61 [b]	2.43E+04	1.5	1.5	52.8	10.0	317.5	linear
Mirex	6.89 [b]	5.28E+05	4.7	4.7	366.2	10.0	101.3	linear

Table D-1. Continued.

PAH Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Naphthalene	3.45 [g]	1.90E+03	N/A	N/A	N/A	10.0	N/A	linear
Acenaphthylene	4.08 [g]	8.26E+03	2.3	2.3	11.7	10.0	183.7	curvilinear
Acenaphthene	4.22 [g]	1.11E+04	2.7	2.7	13.4	10.0	126.6	curvilinear
Fluorene	4.38 [g]	1.55E+04	3.0	3.0	16.8	10.0	79.8	curvilinear
Phenanthrene	4.46 [g]	1.82E+04	3.9	3.9	15.2	10.0	72.4	curvilinear
Anthracene	4.54 [g]	2.12E+04	3.0	3.0	23.1	10.0	158.7	linear
Fluoranthene	5.20 [g]	6.83E+04	4.3	4.3	51.7	10.0	110.7	linear
Pyrene	5.30 [g]	8.00E+04	5.1	5.1	51.1	10.0	93.4	linear
Benz[*a*]anthracene	5.91 [g]	1.89E+05	3.6	3.6	171.2	10.0	132.3	linear
Chrysene	5.61 [g]	1.27E+05	4.0	4.0	103.1	10.0	119.0	linear
Benzo[*b*]fluoranthene	5.78 [g]	1.60E+05	3.2	3.2	162.7	10.0	148.8	linear
Benzo[*k*]fluoranthene	6.20 [g]	2.68E+05	3.4	3.4	256.8	10.0	140.1	linear
Benzo[*a*]pyrene	6.35 [g]	3.16E+05	3.5	3.5	294.3	10.0	136.1	linear

Table D-1. Continued.

PAH Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Indeno[*1,2,3-cd*]pyrene	6.75 [g]	4.68E+05	3.3	3.3	462.1	10.0	144.3	linear
Dibenzo[*a,h*]anthracene	6.51 [g]	3.73E+05	2.3	2.3	528.3	10.0	207.0	linear
Benzo[*g,h,I*]perylene	6.90 [g]	5.33E+05	1.9	1.9	913.4	10.0	250.6	linear

[a] OCs and PAHs are listed in order of their chromatographic elution on a DB-35Ms and a DB-5 GC column. Equation 3-11 (linear model) was used in cases where a compound's log $K_{ow} > 6$. As mentioned earlier, this calculator applies only to SPMDs, which conform to the surface area-to-volume ratio of a standard SPMD. If multiple log K_{ow} values were found in the literature, a mean value was selected using the t-test at 95% confidence for rejection of outliers.
[b] Mackay *et .al.* (1997).
[c] Oliver *et .al.* (1985).
[d] Simpson *et .al.* (1995).
[e] Veith *et .al.* (1979).
[f] Syracuse Research Corporation, On-Line Log K_{ow} Estimator (KowWin), http://esc.syrres.com/interkow/logkow.htm.
[g] Huckins *et .al.* (1999).
[h] Linear, curvilinear, and equilibrium models were used for computation of water concentrations.

Table D-2. Estimate of Water Concentration from SPMDs Exposed to 18 °C Water for 21 Days Using the Excel® Calculator.

OC Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Hexachlorobenzene	5.71 [b]	1.45E+05	3.6	3.6	131.6	10.0	132.3	linear
Pentachloroanisole	5.48 [c,f]	1.05E+05	4.9	4.9	69.8	10.0	97.2	linear
α-BHC	3.86 [b]	5.06E+03	1.4	1.4	11.8	10.0	299.0	curvilinear
Lindane	3.71 [b]	3.57E+03	1.1	1.1	10.6	10.0	446.4	curvilinear
β-BHC	3.86 [b]	5.06E+03	N/A	N/A	N/A	10.0	N/A	linear
Heptachlor	5.19 [b]	6.72E+04	N/A	N/A	N/A	10.0	N/A	linear
d-BHC	4.12 [b]	9.01E+03	N/A	N/A	N/A	10.0	N/A	linear
Dacthal	4.26 [b]	1.21E+04	1.8	1.8	21.9	10.0	264.6	linear
Oxychlordane	5.48 [b]	1.05E+05	2.4	2.4	142.6	10.0	198.4	linear
Heptachlor Epoxide	4.51 [b]	2.00E+04	3.6	3.6	18.1	10.0	58.7	curvilinear
trans-Chlordane	5.38 [b,d,e,f]	9.05E+04	3.1	3.1	95.1	10.0	153.6	linear
trans-Nonachlor	6.35 [d,f]	3.16E+05	2.8	2.8	367.9	10.0	170.1	linear
o,p'-DDE	5.56 [b]	1.18E+05	3.7	3.7	103.9	10.0	128.7	linear
cis-Chlordane	5.38 [b,d,e,f]	9.05E+04	2.8	2.8	105.2	10.0	170.1	linear

Table D-2. Continued.

OC Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Endosulfan	3.78 [b]	4.21E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-DDE	6.14 [b]	2.50E+05	6.9	6.9	118.1	10.0	69.0	linear
Dieldrin	4.60 [b]	2.38E+04	4.0	4.0	19.4	10.0	47.2	curvilinear
o,p'-DDD	6.08 [b]	2.33E+05	3.3	3.3	230.1	10.0	144.3	linear
Endrin	4.63 [b]	2.52E+04	4.9	4.9	16.8	10.0	49.0	curvilinear
cis-Nonachlor	6.20 [d,f]	2.68E+05	3.0	3.0	291.0	10.0	158.7	linear
o,p'-DDT	5.59 [b]	1.23E+05	4.3	4.3	93.3	10.0	110.7	linear
p,p'-DDD	5.75 [b]	1.54E+05	3.8	3.8	131.6	10.0	125.3	linear
Endosulfan-II	3.50 [f]	2.15E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-DDT	5.47 [b]	1.04E+05	5.6	5.6	60.2	10.0	85.0	linear
Endosulfan Sulfate	3.64 [f]	3.02E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-Methoxychlor	4.61 [b]	2.43E+04	6.2	6.2	12.8	10.0	59.7	curvilinear
Mirex	6.89 [b]	5.28E+05	5.0	5.0	344.2	10.0	95.2	linear

Table D-2. Continued.

PAH Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Naphthalene	3.45 [g]	1.90E+03	0.9	0.9	6.9	10.0	988.7	curvilinear
Acenaphthylene	4.08 [g]	8.26E+03	1.4	1.4	19.2	10.0	137.1	curvilinear
Acenaphthene	4.22 [g]	1.11E+04	2.3	2.3	15.8	10.0	115.4	curvilinear
Fluorene	4.38 [g]	1.55E+04	1.7	1.7	29.7	10.0	280.1	linear
Phenanthrene	4.46 [g]	1.82E+04	3.4	3.4	17.4	10.0	66.5	curvilinear
Anthracene	4.54 [g]	2.12E+04	3.6	3.6	19.2	10.0	53.3	curvilinear
Fluoranthene	5.20 [g]	6.83E+04	4.6	4.6	48.4	10.0	103.5	linear
Pyrene	5.30 [g]	8.00E+04	5.2	5.2	50.1	10.0	91.6	linear
Benz[*a*]anthracene	5.91 [g]	1.89E+05	3.6	3.6	171.2	10.0	132.3	linear
Chrysene	5.61 [g]	1.27E+05	5.1	5.1	80.9	10.0	93.4	linear
Benzo[*b*]fluoranthene	5.78 [g]	1.60E+05	3.4	3.4	153.1	10.0	140.1	linear
Benzo[*k*]fluoranthene	6.20 [g]	2.68E+05	4.0	4.0	218.3	10.0	119.0	linear
Benzo[*a*]pyrene	6.35 [g]	3.16E+05	4.3	4.3	239.5	10.0	110.7	linear

Table D-2. Continued.

PAH Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Indeno[*1,2,3-cd*]pyrene	6.75 [g]	4.68E+05	4.2	4.2	363.1	10.0	113.4	linear
Dibenzo[*a,h*]anthracene	6.51 [g]	3.73E+05	3.3	3.3	368.2	10.0	144.3	linear
Benzo[*g,h,I*]perylene	6.90 [g]	5.33E+05	2.4	2.4	723.1	10.0	198.4	linear

[a] OCs and PAHs are listed in order of their chromatographic elution on a DB-35Ms and a DB-5 GC column. Equation 3-11 (linear model) was used in cases where a compound's log K_{ow}> 6. As mentioned earlier, this calculator applies only to SPMDs, which conform to the surface area-to-volume ratio of a standard SPMD. If multiple log K_{ow} values were found in the literature, a mean value was selected using the t-test at 95% confidence for rejection of outliers.
[b] Mackay *et .al.* (1997).
[c] Oliver *et .al.* (1985).
[d] Simpson *et .al.* (1995).
[e] Veith *et .al.* (1979).
[f] Syracuse Research Corporation, On-Line Log K_{ow} Estimator (KowWin), http://esc.syrres.com/interkow/logkow.htm.
[g] Huckins *et .al.* (1999).
[h] Linear, curvilinear, and equilibrium models were used for computation of water concentrations.

Table D-3. Estimate of Water Concentration from SPMDs Exposed to 26 °C Water for 60 Days Using the Excel® Calculator.

OC Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Hexachlorobenzene	5.71 [b]	1.45E+05	7.6	7.6	62.3	10.0	21.9	linear
Pentachloroanisole	5.48 [c,f]	1.05E+05	14.0	14.0	24.4	10.0	16.6	curvilinear
α-BHC	3.86 [b]	5.06E+03	4.0	4.0	4.1	10.0	421.5	equilibrium
Lindane	3.71 [b]	3.57E+03	2.3	2.3	5.1	10.0	597.3	equilibrium
β-BHC	3.86 [b]	5.06E+03	1.6	1.6	10.3	10.0	421.5	equilibrium
Heptachlor	5.19 [b]	6.72E+04	9.9	9.9	22.1	10.0	26.9	curvilinear
d-BHC	4.12 [b]	9.01E+03	N/A	N/A	N/A	10.0	N/A	linear
Dacthal	4.26 [b]	1.21E+04	2.0	2.0	19.7	10.0	154.7	curvilinear
Oxychlordane	5.48 [b]	1.05E+05	7.2	7.2	47.5	10.0	11.8	curvilinear
Heptachlor Epoxide	4.51 [b]	2.00E+04	12.9	12.9	5.1	10.0	106.5	equilibrium
trans-Chlordane	5.38 [b,d,e,f]	9.05E+04	9.0	9.0	32.7	10.0	17.0	curvilinear
trans-Nonachlor	6.35 [d,f]	3.16E+05	8.5	8.5	121.2	10.0	19.6	linear
o,p'-DDE	5.56 [b]	1.18E+05	8.8	8.8	43.7	10.0	11.1	curvilinear
cis-Chlordane	5.38 [b,d,e,f]	9.05E+04	9.4	9.4	31.4	10.0	17.3	curvilinear

Table D-3. Continued.

OC Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Endosulfan	3.78 [b]	4.21E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-DDE	6.14 [b]	2.50E+05	14.1	14.1	57.8	10.0	11.8	linear
Dieldrin	4.60 [b]	2.38E+04	6.3	6.3	12.3	10.0	89.5	equilibrium
o,p'-DDD	6.08 [b]	2.33E+05	7.6	7.6	99.9	10.0	21.9	linear
Endrin	4.63 [b]	2.52E+04	10.6	10.6	7.8	10.0	84.5	equilibrium
cis-Nonachlor	6.20 [d,f]	2.68E+05	6.8	6.8	128.4	10.0	24.5	linear
o,p'-DDT	5.59 [b]	1.23E+05	4.6	4.6	87.2	10.0	36.2	linear
p,p'-DDD	5.75 [b]	1.54E+05	8.7	8.7	57.5	10.0	7.2	curvilinear
Endosulfan-II	3.50 [f]	2.15E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-DDT	5.47 [b]	1.04E+05	5.5	5.5	61.3	10.0	30.3	linear
Endosulfan Sulfate	3.64 [f]	3.02E+03	N/A	N/A	N/A	10.0	N/A	linear
p,p'-Methoxychlor	4.61 [b]	2.43E+04	2.8	2.8	28.3	10.0	67.7	curvilinear
Mirex	6.89 [b]	5.28E+05	10.1	10.1	170.4	10.0	16.5	linear

Table D-3. Continued..

PAH Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Naphthalene	3.45 [g]	1.90E+03	0.5	0.5	12.4	10.0	1123.8	equilibrium
Acenaphthylene	4.08 [g]	8.26E+03	1.7	1.7	15.8	10.0	239.5	curvilinear
Acenaphthene	4.22 [g]	1.11E+04	2.4	2.4	15.1	10.0	179.2	curvilinear
Fluorene	4.38 [g]	1.55E+04	2.8	2.8	18.0	10.0	124.1	curvilinear
Phenanthrene	4.46 [g]	1.82E+04	4.6	4.6	12.9	10.0	117.4	equilibrium
Anthracene	4.54 [g]	2.12E+04	3.8	3.8	18.2	10.0	90.2	curvilinear
Fluoranthene	5.20 [g]	6.83E+04	7.2	7.2	30.9	10.0	23.1	curvilinear
Pyrene	5.30 [g]	8.00E+04	7.9	7.9	33.0	10.0	19.1	curvilinear
Benz[*a*]anthracene	5.91 [g]	1.89E+05	5.5	5.5	112.1	10.0	30.3	linear
Chrysene	5.61 [g]	1.27E+05	7.4	7.4	55.8	10.0	8.9	curvilinear
Benzo[*b*]fluoranthene	5.78 [g]	1.60E+05	3.6	3.6	144.6	10.0	46.3	linear
Benzo[*k*]fluoranthene	6.20 [g]	2.68E+05	6.2	6.2	140.8	10.0	26.9	linear
Benzo[*a*]pyrene	6.35 [g]	3.16E+05	5.6	5.6	183.9	10.0	29.8	linear

Table D-3. Continued.

PAH Compounds [a]	Log K_{ow}	K_{SPMD}	Laboratory $R_{s\text{-}cal}$ (L/d)	PRC corrected R_s (L/d)	Theoretical t_{50}	Total Analyte (ng/SPMD)	Estimated Water Conc. (pg/L)	Model Used [h]
Indeno[*1,2,3-cd*]pyrene	6.75 [g]	4.68E+05	4.8	4.8	317.7	10.0	34.7	linear
Dibenzo[*a,h*]anthracene	6.51 [g]	3.73E+05	3.1	3.1	392.0	10.0	53.8	linear
Benzo[*g,h,I*]perylene	6.90 [g]	5.33E+05	2.5	2.5	694.2	10.0	66.7	linear

[a] OCs and PAHs are listed in order of their chromatographic elution on a DB-35Ms and a DB-5 GC column. Equation 3-11 (linear model) was used in cases where a compound's log $K_{ow} > 6$. As mentioned earlier, this calculator applies only to SPMDs, which conform to the surface area-to-volume ratio of a standard SPMD. If multiple log K_{ow} values were found in the literature, a mean value was selected using the t-test at 95% confidence for rejection of outliers.
[b] Mackay *et .al.* (1997).
[c] Oliver *et .al.* (1985).
[d] Simpson *et .al.* (1995).
[e] Veith *et .al.* (1979).
[f] Syracuse Research Corporation, On-Line Log K_{ow} Estimator (KowWin), http://esc.syrres.com/interkow/logkow.htm.
[g] Huckins *et .al.* (1999).
[h] Linear, curvilinear, and equilibrium models were used for computation of water concentrations.

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