Laboratory Studies of the Use of Semipermeable Membrane Devices (SPMDs) as Passive Water Samplers of Polyaromatic Hydrocarbon (PAH) Priority Pollutants

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EXECUTIVE SUMMARY

Because of the global use of petroleum based products, polycyclic aromatic hydrocarbons (PAHs) are one of the most widely distributed groups of environmental contaminants. Consequently, defining their concentrations and bioavailability in a wide array of ecosystems is a critical step in assessing the potential ecological risk of these contaminants. However, determining the bioavailable portion of PAH residues (typically a small but variable fraction of the total residue) present in environmental water samples is problematic due to the difficulty of separating dissolved residues from those associated with a wide range of concentrations associated with particulate and dissolved organic carbon. Moreover, the use of sentinel organisms (e.g., fish, bivalves, etc.) to sample the bioavailable fraction of PAHs is often complicated by extensive metabolism and excretion (particularly fish) or selective depuration (bivalves) of the residues present in the water.

Scientists at the National Biological Survey's Midwest Science Center (MSC) have developed a passive sampler -- the semipermeable membrane device (SPMD) -- that appear to overcome the limitations associated with analysis of excised water samples and sentinel organisms. The SPMD is designed to mimic the bioconcentration (i.e. respiratory uptake) of contaminants by biota. The SPMD approach offers the high precision and

reproducibility of analytical chemistry methods with none of their mechanical short comings, yet acts as a "in situ" integrator of contaminants similar to biomonitoring organisms.

The subject of this report is the laboratory portion of the overall research process necessary to validate the use of SPMDs as monitors of PAHs in aquatic environments. The flow-through (i.e., kinetic) and static (i.e., equilibrium) studies (using the EPA's priority pollutant PAHs) conducted during this project are critical to the development and parameterization of the algorithms for calculating ambient water concentrations of PAHs from their respective levels in SPMDs. Three flow-through 21 day diluter studies and a 56 day static exposure study were completed. The diluter studies were conducted at environmentally realistic concentrations of 1 ng/L, 10 ng/L and 100 ng/L and at 10^0C, 18^0C, and 26^0C. Samples were taken temporally at days 4,7,14 and 21 and the rate of SPMD uptake determined. The 56 day static exposures were conducted at 24^0C with an initial PAH concentration of about 1 μg/L. Samples were taken temporally at days 1,4,7,14,28 and 56 to determine the steady state concentration factors of PAHs in SPMDs. Approximately 1000 samples were analyzed for residues of the 16 priority pollutant PAHs (i.e., 16,000 individual determinations) during the course of this project.

Briefly, the results of these studies indicate the following:

- SPMDs perform well as in situ integrative samplers of aqueous PAHs

- Screening tests indicate that salinity has little effect on PAH uptake rate

- Concentration factors (static exposure) of several PAHs in SPMD lipids exceeded 400,000

- Uptake rates increase with PAH octanol/water partition coefficients up to about 200,000 and then decline as molecular size limits SPMD membrane permeability

- Only relatively small differences in PAH uptake rates were observed at exposure temperatures of 10^0C, 18^0C and 26^0C

- Sampling rates appear to be relatively independent of PAH water concentrations within the concentration range tested

- For PAHs with octanol/water partition coefficients greater than 100,000, sampling rates remained relatively constant during the 21 day flow-through exposure

- The marked similarity of SPMD PAH uptake rates and contaminant uptake rates across fish gills (in both cases, relative to log octonal-water partition coefficients) indicates that SPMDs do mimic the bioconcentration process of nonmetabolized compounds

The results of these studies provide a laboratory data base adequate to proceed to the validation of SPMDs as monitors of PAHs in environmental settings. No other technology or procedure appears to offer as ready access to the concentrations of bioavailable environmental contaminants.

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INTRODUCTION

In order to meet guidelines designed to protect fish and wildlife resources, resource managers, regulatory agencies, and industry must have knowledge of the identity and average concentrations of bioavailable pollutants in effluents entering surface waters (i.e. exposure concentration assessment). This information is a fundamental part of assessing the effects of contaminated effluents on fish, wildlife, and ultimately humans. Unfortunately, these key data are often unavailable to researchers and decision makers because of limitations in analytical and biomonitoring approaches. These limitations include: 1) current use of non-integrative sampling methods (grab or excised samples, often reflect contaminant residue composition only at the moment or window of sampling) for monitoring hydrophobic pollutants in water which often fail to detect rapidly changing or episodic contamination events; 2) standard analytical methods for the determination of trace-level ($\leq$ 0.1 μg/L) nonpolar organics in water are problematic because of the difficulties encountered in collecting and extracting large volumes of water, changes in water quality associated with sampling and handling and in compensating for losses of contaminants due to filtration, volatilization, and sorption on non-equilibrated surfaces; 3) integrative biomonitors, such as caged fish and mussels, are often difficult to find free of contamination, cannot be used in highly toxic environments, may

not concentrate many contaminants due to their ability to actively depurate or metabolize certain classes of pollutants, and biomonitoring organisms usually have seasonal, lifestage, sex and species variations in the quantity and quality of their lipids which affects the bioconcentration of contaminants.

Improvements in traditional contaminant monitoring methods may partly address these problems, however, new approaches are needed that combine the positive attributes of commonly used analytical chemistry (i.e. accurate and precise) with that of biomonitoring approaches (i.e. integration of bioavailable aqueous residues). What is critically needed in this respect is an environmental dosimetry approach that permits assessment of fish and wildlife exposure to contaminants in a manner similar to that employed in personal dosimeters for determining time-weighted occupational exposure in humans.

Recently, the National Biological Survey's Midwest Science Center developed a semipermeable polymeric membrane device (SPMD) containing a thin film of pure lipid (triolein) for <u>in situ</u> passive monitoring of trace aquatic contaminants (1). This conceptually simple device appears to address a number of the aforementioned limitations in analytical and biomonitoring techniques for aqueous contaminants and is based on concepts similar to widely accepted passive air monitors. Also, the passive partitioning process mediating SPMD uptake mimics the

tendency of aquatic life to bioconcentrate trace organic contaminants from water. At equilibrium, concentration factors of aqueous contaminants in SPMDs are approximately equal to their respective octonal-water partition coefficients (a commonly used and often available parameter used to predict bioconcentration). The devices will enable investigators to measure the presence and bioavailability of organic contaminants in diverse aquatic habitats. SPMDs have significant statistical advantages over traditional contaminant assessments due to their reproducibility and the ease of data interpretation. Further, they provide a relative index of pollution severity, have the ability to sequester contaminants from episodic spills, will concentrate pollutants often metabolized by aquatic organisms, and can be used in surface water, sediment, and groundwater environs.

SPMDs are constructed of virgin (no additives) low-density layflat polyethylene tubing enclosing a thin film of 95% or 99% pure triolein (major neutral lipid in many aquatic organisms). The membrane controls contaminant uptake rate (2); a key factor in designing a precision sampling device. The thin film design is used to maximize surface area (membrane) to lipid volume ratio which in turn allows high contaminant sampling rates for a passive sampler. Typically, $\simeq$ 1 g of triolein is placed in a layflat tube which, after heat sealing into a loop configuration, is ready for field deployment. Both lipid and polyethylene tubing lots are extracted and analyzed for interfering substances

prior to use. When membrane impurities are found, a simple organic solvent dialysis step will generally remove the interfering substance. Because the membrane prevents microbial attack of lipid, extended field exposures of SPMDs can be conducted. After exposures, SPMDs are returned to the laboratory in clean gas-tight containers on ice (contaminants in SPMDs can be preserved indefinitely by freezing the intact samplers). Organic contaminants are recovered from intact field deployed SPMDs by organic solvent dialysis (3) and this procedure results in very low lipid carryover in the analyte fraction. The dialysis process is simpler than the grinding and extraction of animal tissues. Subsequent class fractionation and instrumental analysis of dialysates depends on the types and complexity of contaminant residues sequestered in SPMDs.

This research is designed to provide key uptake kinetics data (includes temperature and concentration effects) necessary to further develop the algorithm for using SPMDs as quantitative monitors of bioavailable polyaromatic hydrocarbons (PAHs) in water. Although SPMDs have been successfully deployed in a number of field environments, laboratory research to elucidate the effects of temperature, and concentration on SPMD uptake rates is required to interpret field data. Continuous flow laboratory exposures (constant concentration) of SPMDs to contaminant mixtures at several temperatures are required to generate the quantitative data to optimize and parameterize

models to predict PAH water concentrations. Numerous static exposures of SPMDs to PAHs are also required to determine the equilibrium partition coefficients needed to select the appropriate mathematical model for estimation of water concentrations from SPMD concentrations. With this data, the SPMD approach should provide a relevant estimate of aqueous contaminant dose to aquatic life, thus contributing toward establishing or disproving potential causal links to observed environmental effects on fish, wildlife, and the ecosystems supporting them.

The compounds examined in this research were the priority pollutant PAHs:

acenaphthene
acenaphthylene
anthracene
benz(a)anthracene
benzo(a)pyrene
benzo(b)fluoranthene
benzo(g,h,i)perylene
benzo(k)fluoranthene
chrysene
dibenz(a,h)anthracene
fluoranthene
fluorene

indeno(1,2,3-c,d)pyrene

naphthalene

phenanthrene

pyrene

These contaminants were chosen by the U.S. EPA as representative of the PAH contamination entering the environment.

An estimated 230,000 metric tons of PAHs enter the global aquatic environment annually from spills and seeps of petroleum, discharges from domestic and industrial sources, aerial transport, and biosynthesis (4). Because of the continuing need for fossil fuels, it seems unlikely that inputs from energy-related activities will greatly decrease during the next several decades. Consequently, potential effects of PAHs on aquatic environments will continue to be of concern to regulatory and resource management agencies and to the general public.

The heterogenous nature of aquatic PAH residues (5,6) confounds efforts to understand which PAHs may pose a threat to aquatic ecosystems and their sources. Moreover, the propensity of fish -- the sentinel organisms of choice for monitoring contaminant residues in freshwater environments -- to rapidly biotransform PAHs through activation of the mixed function oxidase enzyme system severely limits possible correlation of PAH concentrations in fish tissue with exposure concentrations in water (7). For

marine environments, mussels and other bivalve molluscs are the biomonitoring organisms of choice. However, all organisms have active transport (uptake and depuration) systems affecting steady state concentrations of chemicals and even bivalves have the ability to metabolize some hydrocarbons (especially lower molecular weight compounds). Ingestion of particulates with sorbed chemicals can also complicate interpretation of bivalve data (8). Thus, their tissue concentrations often may not reflect the true concentrations of bioavailable compounds in water. Measurement of toxicologically significant PAH residues in water by conventional analytical techniques is problematic because of their low levels.

These factors, in combination with the continuum of physicochemical properties of PAHs (5) renders the determination of bioavailable water concentrations (i.e. an estimation of organism exposure) of PAHs a formidable task. For these reasons SPMD technology appears to be ideally suited for the analysis of PAHs.

THEORY AND MODEL DEVELOPMENT

Because SPMD sampling rate and equilibrium capacity varies with PAH physicochemical properties, analyte concentrations in exposed SPMDs may represent any one of three possible regions of an

uptake curve (linear, curvilinear and asymptotic). Overall SPMD uptake of aqueous PAHs can be described by

$$C_L = C_W K_{LW} (1-\exp[-k_U t]) \quad (1)$$

where C_L and C_W are analyte concentrations in the triolein and water, K_{LW} is the equilibrium lipid/water partition coefficient (approximated by the octonal-water partition coefficient [K_{OW}] of a compound) and k_U is the overall uptake rate constant. The capacity of the SPMD to sequester an analyte is set by the K_{LW} value (model amplitude) and k_U, the overall uptake rate constant, is $k_O A K_{ML}/V_L$ where k_O is the mass transfer coefficient of an analyte into the SPMD triolein (m/h), A is membrane surface area (m^2), K_{ML} is the membrane-lipid partition coefficient and V_L is the lipid volume (m^3). Use of equation 1 to predict C_W requires multiple determinations of C_L through time or kinetics data. The number of estimated parameters, e.g. C_W and k_U should generally be no more than half the number of C_L values measured. Also note that because membrane fouling often occurs during environmental exposures, SPMD uptake may be biphasic (nonexponential) in nature resulting in poor (exponential) model fits to the data.

Assuming equilibrium between the SPMD lipid and exposure water is not approached for a particular analyte (i.e. strictly speaking $C_L/C_W << K_{LW}$ but $\leq 0.5\ K_{LW}$ or one half-time has not been exceeded), then for a constant temperature

$$C_L = C_W K_{MW} k_O A t / V_L \qquad (2)$$

where K_{MW} is the membrane/water partition coefficient. Here the group $K_{MW} k_O A$ is the SPMD effective daily sampling rate (R_S) for a particular analyte, i.e. m^3/d or L/d. The use of equation 2 to estimate analyte C_W requires that the SPMD is still in the linear sampling phase or that the average sampling rate during the interval of exposure is known. When $C_L/C_W \simeq K_{LW}$, the exponential term in equation 1 becomes negligible and

$$C_L = C_W K_{LW} \qquad (3)$$

This is the region in which C_L reaches equilibrium with C_W. Then if K_{LW} is known, the water concentration C_W can be determined from C_L.

If organic solvent dialysis is used to extract contaminant residues sequestered by SPMDs, the recovered analyte mass is

representative of both the lipid and membrane phases of the SPMD. In some cases the amount of analyte in the membrane can be 40% of the total SPMD residue. The following equation can be applied to estimate C_L from the total amount of residue recovered by dialysis:

$$C_L = A_{MD}/(M_L + K_{ML}M_M) \qquad (4)$$

where A_{MD} is the total mass of the analyte in the dialysate, M_L is the mass of the triolein, K_{ML} was defined earlier and M_M is the mass of the membrane.

Both temperature and biofouling can affect the R_S of an SPMD. The possible reduction in analyte R_S by biofouling was assessed earlier by recovering heavily fouled SPMDs (58 day exposure) from the Upper Mississippi River and exposing them along with fresh SPMDs to constant aqueous concentrations of phenanthrene (9). The reduction in SPMD uptake due to fouling ranged from about 25 to 40% of the R_S value. Temperature affects molecular diffusion and polymer free volume, and thus higher temperatures often results in larger R_S values for some analytes. The use of a permeability reference standard (i.e. a noninterfering compound(s) with moderate SPMD fugacity added to the lipid just prior to deployment) as a method to correct SPMD R_S values for

the effects of variable temperature and fouling, is underway at MSC with selected deuterated PAHs.

At this stage of SPMD development, the data recommended for generating SPMD estimates of the average PAH concentrations in an exposure media include: 1) mean temperature during the exposure period, 2) visual observations of the extent of membrane biofouling, 3) R_S values (possible temperature and concentration effects factored in) of contaminants of interest for the exposure duration used and 4) measured C_L values or total analyte mass sequestered in the SPMD (here the K_{ML} partition coefficient must be known).

EXPERIMENTAL

LABORATORY PAH EXPOSURES:

Three separate exposure studies were completed during this project. Laboratory deep well water of known quality (2) was used in two studies but was amended for the third study (marine-freshwater comparisons). The first study consisted of exposing SPMDs to the priority pollutant PAHs in a flow-through freshwater dilutor system. Nominal PAH water concentrations in flow-through exposures were 0 ng/L (control), 1 ng/L, 10 ng/L and 100 ng/L. These exposures were conducted at $10^{O}C$, $18^{O}C$, and $26^{O}C$.

SPMDs (N=3) were sampled on days 0 and 4, 7, 14 and 21. Water samples were collected on days 0 and 21. The SPMDs in the flow-through diluters were treated weekly with Sanaqua®, a 7.5% aqueous solution of didecyl dimethyl ammonium chloride, to minimize periphyton growth. Details of these uptake kinetics studies are presented in the attached protocol, P-93-33-01 (Appendix A).

The second exposure study consisted of static chambers containing the priority pollutant PAHs in freshwater (mean exposure temperature was $24^{0}C$). Each chamber with the exception of controls was dosed (single application) with 0.91 μg of each PAH, as the target concentration was 1 μg/L (note that this nominal level exceeds the water solubility of dibenz[a,h]anthracene, indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene). Three replicate SPMDs were sampled on days 1, 4 , 7, 14, 28 and 56. Water samples (approximately 900 mL) were taken at each SPMD sampling time. The triolein and the polyethylene membrane of each SPMD were analyzed separately in order to define the lipid/membrane partition coefficient. Details of this static uptake study is presented in the attached protocol, P-94-33-02 (Appendix A).

Because of the large differences in the salinities of fresh, estuarine, and marine waters it is critical to examine the potential effect of this variable on the uptake of PAHs by SPMDs.

Consequently, a third study was conducted to obtain data on the uptake of PAHs by SPMDs under varying salinity regimes. This screening study consisted of separate 28 day static exposures of SPMDs to radiolabeled model PAHs. Water quality used for both model PAH exposures was representative of freshwater, estuarine, and ocean environments. Deep well water was reconstituted with Instant Ocean® at the 11 ppt (estuarine) and 33 ppt (marine) levels for use in this study (mean exposure temperature was $18^{0}C$). The model radiolabeled (ring-uL-^{14}C) PAHs chosen for the study were phenanthrene and dibenz(a,h)anthracene. These compounds are representative of both the smaller molecular weight PAHs (phenanthrene = 3 rings) and the higher molecular weight PAHs (dibenz(a,h)anthracene = 5 rings). The SPMDs (N=3) were sampled on days 4, 7, 14 and 28 of the exposure. A mass balance approach was used to define the distribution of the PAH residues among all components (i.e., lipid, membrane, water, container wall, etc.) of the exposure chambers.

MATERIALS AND REAGENTS:

All solvents used in this work were pesticide grade or equivalent. Triolein (1,2,3-Tri-[(Cis)-9-octadecenoyl] glycerol) was purchased from Sigma Chemical Co., St. Louis, MO., USA. The triolein used was 95% pure and was not further purified before use because of its low PAH background. It was ampulated and

stored at -20^{0}C until use. Low-density polyethylene (PE) tubing (layflat; 2.54 cm wide, no. 940, obtained from Brentwood Plastics, Brentwood, MO, USA was untreated, pure PE, no slip additives, stabilizers, etc.) was used to prepare the SPMDs. The wall thickness of the lot used ranged from 84 to 89 μm. The heat-sealer used in making the SPMDs was an Impulse Sealer model no. AIE-100 from American International Electric, Santa Fe Spring, CA., USA. Gold fluorescent lamps were obtained from Phillips Lighting Corp., Bloomfield, NJ, USA. Except when protected by amber glassware, samples and standard materials were kept in darkness or handled under gold lights to prevent photodegradation.

PREPARATION OF SPMDS:

All SPMDs were assembled in a clean room (vapor phase PAHs in ambient air were removed by continuous charcoal filtration and exposure to numerous SPMDs suspended in the clean room). Layflat PE tubing was cut into 46 cm segments. Contaminants were removed from the PE segments prior to making SPMDs by batch-extracting the segments with hexane. Following the batch-extraction procedure, the PE segments were air-dried and subsequently stored in sealed, amber-glass jars maintained in the clean room (described earlier) until used to make SPMDs.

We pipetted 0.5 mL (flow-through and static [salinity treatments] exposures) and 0.1 mL (static fresh water exposures) of triolein into one end of each segment of PE tubing. The segments were laid on a solvent-cleaned, flat surface, and the triolein was squeezed toward the opposite end of the tubing until it formed a thin film through the central portion of each PE segment. Because triolein can interfere with heat sealing of the membrane, care must be taken to avoid spreading triolein near the ends (about 2 cm of each tubing end free of triolein) of the tubing. Each end of the tubing was then sealed by forming three molecular welds (heat seals) at each end. Details of the preparation procedure are given in the attached standard operating procedure (SOP) B5.217 (Appendix B). Also, see Appendix B for SOPs related to all analytical procedures used in this work. As configured for this study, each SPMD had 198 cm^2 of surface area.

SAMPLE PROCESSING AND RESIDUE ENRICHMENT:

The volumes of water sampled from flow-through exposures were 8 L for the control and 1 ng/L exposure chambers, 4L for the 10 ng/L exposure chamber, and 2 L for the 100 ng/L chamber. They were collected on days 0 and 21. Each sample was extracted three times with methylene chloride (100 mL per extract); the organic extracts of individual samples were combined, dried over sodium sulfate, reduced in volume to 1 mL, and subsequently analyzed by gas chromatography photoionization detection (GC-PID) for PAH

residues. Water samples (<1L volume) from both static exposure studies were extracted similarly and concentrated as described for flow-through exposure samples. Extracts from the salinity screening test were analyzed by liquid scintillation counting whereas extracts containing priority pollutant PAHs were analyzed as described for flow-through exposures.

Processing of SPMDs involved the removal of exterior periphytic growth, organic solvent dialysis (10), treatment by high performance gel permeation chromatography (HPGPC) and potassium silicate chromatography (KS), and subsequent analysis by GC-PID for PAH residues. Removal of the surficial periphytic layer was accomplished by placing the SPMDs (Note: The SPMD membrane should not be touched with bare hands prior to the surficial cleaning) in a 500-mL, wide mouth, amber glass jar equipped with a Teflon-lined cap. Hexane (100 mL) was added to the glass jar the lid was tightly closed, and the jar was shaken for approximately 10 seconds (caution, extraction times > 10 seconds may recover some PAHs from the membrane) and the hexane was discarded. Following extraction of the surficial layer, the SPMDs were placed in a stainless steel pan and rinsed with copious amounts of clean running water. The SPMDs were subsequently submerged in a glass tank containing 1 N HCl for approximately 30 seconds. Following the HCl treatment, they were again rinsed with running water to remove residual acid. All water on membrane surfaces was removed using brief rinses of

acetone followed by isopropanol. The SPMDs were allowed to air dry for approximately 5 minutes (Note: Unless the atmosphere is organic contaminant free, air drying may result in uptake of airborne contaminants) by placing them on a piece of solvent-rinsed aluminum foil in a clean room free of organic contaminants.

Dialysis of SPMDs was accomplished using 8 oz (0.24 L) jars with solvent-rinsed aluminum foil under the screw-type lids to create a tight seal. Each 0.5 mL triolein SPMD was dialyzed with 75 mL hexane. In all cases, SPMDs were allowed to dialyze for 48 hours at 18^{0}C. Subsequently, SPMDs were removed from the jar, and the dialysate was quantitatively transferred to round-bottom flasks. Then the dialysates were reduced to approximately 5 mL using a rotary evaporation system. The dialysates were quantitatively transferred to test tubes using several 1-2 mL hexane rinses and further reduced in volume (≈0.5 mL) using high-purity nitrogen.

Concentrated dialysates and lipid rinses (SPMDs from static fresh water exposures) were quantitatively transferred to one mL conical autoinjector vials using methylene chloride; a nonane keeper was added (about 0.1 mL) and the methylene chloride was removed under a stream of high purity nitrogen. Final volumes were adjusted to approximately 0.95 mL using nonane. The resulting sample solutions were fractionated by HPGPC. The GPC instrumentation included the following modules: Perkin-Elmer

series 410 solvent delivery system with a Perkin-Elmer ISS-200 autoinjector system (Perkin-Elmer Co., Norwalk, CN), and ISCO Foxy 200 fraction collector (ISCO, Inc., Lincoln, NE), and a 7.8 mm i.d. x 100 mm, 10 μm (10 nm pore size) Phenogel® GPC column (Phenomenex, Inc., Torrance, CA). The mobile phase consisted of hexane/methylene chloride (80/20, V/V) delivered at a flow rate of 2.5 mL/min. Prior to sample chromatography, the GPC system was calibrated by injecting benzene and monitoring its retention. The collect cycle (26-69 mL) was initiated at the beginning of the elution of benzene; all previous eluate (0-26 mL) constituted the excluded (dump) fraction. The dump fraction contained co-dialyzed lipid components and polyethylene waxes, and the collect fraction contained all priority pollutant PAHs. The sample collect fractions were quantitatively transferred to round-bottom flasks, and reduced to about 5 mL using a rotary evaporation system. Then they were quantitatively transferred to test tubes using several 1-2 mL hexane rinses and further reduced in volume (about 0.5 mL) using high purity nitrogen.

These concentrated HPGPC eluates were quantitatively transferred to a KS column - 3 cm segment of KS overlain with 0.25 cm of anhydrous Na_2SO_4 - and eluted with adequate methylene chloride/hexane (7/93, V/V) to collect 5.5 of eluate. The eluates from the KS columns were evaporated to final volumes of 0.1, 0.5 and 2 mL (1 ng/L, 10 ng/L and 100 ng/L samples, respectively) using high purity nitrogen. The internal standard,

azulene, was added to these final solutions at 100 ng, 500 ng, and 2000 ng, respectively.

Processing of SPMDs from static freshwater exposures involved the analysis of both the membrane and lipid as separate samples. Lipid was rinsed from the inside of SPMDs using multiple washes of hexane (10-20 ml total) and concentrated as described for dialysates. This concentrate was then cleaned up by HPGPC as described earlier. Analytical procedures for SPMD membranes were identical to those used for whole SPMDs.

INSTRUMENTAL ANALYSIS:

SPMD, water and QC sample solutions were analyzed using a Hewlett Packard Model 5890 Capillary GC system (Hewlett Packard, Inc., Palo Alto, CA, USA). Detection was by means of a Perkin Elmer Model PI5202 Photo Ionization Detector (PID, Perkin-Elmer, Inc., Norwalk, CN, USA) operated at 270^{0}C and containing a 9.5 - eV lamp (HNU Systems, Inc., Newton, MA, USA). Injections of 2 μL or 5 μL of each sample solution (2 mL and 0.5 and 0.1 mL final volume) were made in the cool, on-column mode into a 1-m X 0.53-mm (i.d.) deactivated fused silica retention gap (Restek Corp., Belletonte, PA, USA) connected to a 30 m x 0.25mm (i.d.) fused silica capillary GC column with 5% phenyl/95% methylpolysiloxane bonded phase (DB-5; J&W Scientific, Folsom, CA, USA). The analysis was accomplished using the following temperature

program: 10^0C/min. from 60^0C to 110^0C; then 5^0C/min to 200^0C; followed by 4^0C/min. to 290^0C and held at 290^0C for two mins. The GC-PID analyses were calibrated using five levels of the priority pollutant PAHs (0.05 ng/μL to 2.00 ng/μL). Quantitation of PAH residues was accomplished by comparing the response of specific peaks in the sample chromatograms with authentic PAH peaks in the calibration standard.

QUALITY CONTROL (QC):

For all studies and sample matricies (SPMD and water) the method limit of detection (MDL) and method limit of quantitation (MQL) was determined by measuring the GC-PID response of coincident peaks for each analyte from control SPMDs (N=3) or water taken through the entire sample cleanup procedure. The MDL was defined as the mean response in quantitation windows of control samples plus three standard deviations of values so determined. The MQL was defined as the mean plus 10 standard deviations of the control values.

The background associated with diluter control SPMDs and static freshwater control SPMDs was found to increase through time - particularly the peak coincident with fluoranthene. Consequently, MDLs and MQLs were determined for each set of dilutor SPMDs and for each sample day and temperature. The MDLs

and MQLs are presented in QC Tables I A-D, II A-D and III A-D (Appendix C) and for static freshwater SPMDs (samples on days 1,4,7,14,28 and 56) in Tables IV A-B (Appendix C). For those analytes with no coincident response in the SPMD controls, an average noise peak area equivalent to 2 ng was estimated. In these cases, the MDL and MQL are considered to be equivalent.

About 25% of each study sample set consisted of QC samples, i.e. process blanks, controls, spikes, etc. Spiked samples used in diluter studies were generated by fortifying diluter control SPMDs and water with approximately 200 ng of each PAH. For freshwater static exposures, spiked samples consisted of control water and control SPMDs fortified with 900 ng of each PAH.

The spiking level of fortified diluter control samples was verified by analyzing an identical aliquot of the spiking solution by GC-PID. The results of the individual spike verification analyses are presented in Tables V A-C (Appendix C) and are summarized in Table VI (Appendix C). The recoveries (ng/SPMD or ng/L [water]) of the PAH residues from the spiked controls are presented in Tables VII A-D (Appendix C). The percent recoveries are given in Tables VIII A-D (Appendix C).

The spiking level of fortified static control samples was verified by analyzing an identical aliquot of the spiking solution by GC-PID (Table IX, Appendix C). The results of the

analyses (ng/L and ng/SPMD) of the fortified static control water and SPMDs are given in Tables X A-C (Appendix C). The membrane and the lipid were fortified and analyzed separately. The percent recoveries are presented in Tables XI A-C (Appendix C). In general, the recovery of PAHs from spiked SPMDs, through the entire analytical procedure (i.e., dialysis, GPC, KS, etc.), remained constant at about 60%. Naphthalene due to its volatility, was recovered at approximately 30%. Fluoranthene was often non-quantifiable due to a co-eluting unknown representing dilutor background. Higher recoveries of PAHs were observed for fortified SPMD membrane and lipids from freshwater static exposures and were about 80% and 90%, respectively. Recoveries of spiked water samples from dilutor and static freshwater tests averaged about 80 and 90%, respectively.

For each batch (N=12) of SPMD samples from the dilutor and static freshwater studies, an SPMD blank (a freshly prepared SPMD) and a reagent blank (75 mL of hexane) were also taken through the entire procedure (i.e., rotary evaporation, GPC, KS, nitrogen blow down, etc.). Also see Appendix C for other types of QC samples used in these studies. Very few SPMD blanks or reagent blanks (exceptions were elevated naphthalene and fluoranthene in some samples) exhibited peaks coincident with the PAHs greater than those present in the diluter exposed SPMD controls. Thus, no significant PAH contamination was introduced during sample processing and enrichment procedures.

RESULTS AND DISCUSSION

The results of the screening exposures for possible salinity effects on SPMD performance are summarized in Table 1 (data for individual exposures are presented in Tables 2A-C). In the case of phenanthrene, 80% of the available compound was sequestered in SPMDs during the first four days of all treatments. Moreover, the K_{LW} is indistinguishable among the three salinity treatments. After 28 days of exposure the amount of phenanthrene sequestered by the SPMDs was again equivalent in all cases. However, the apparent K_{LW} differed between the three treatments with the K_{LW} for the ocean water being approximately 2 fold greater than the fresh water. This difference in K_{LW}s is due to the approximately 50% lower concentration of phenanthrene in simulated ocean water than in the fresh water. Considering the well known tendency of many organic compounds to "salt out", and the proclivity of PAHs to plate out on surfaces, to undergo biodegradation, and in the case of phenanthrene to volatilize, this difference in the K_{LW}s of SPMDs exposed to different salinity treatment is not unexpected. More importantly, the nearly equivalent amount of phenanthrene sequestered in SPMDs from each treatment suggest that salinity had little or no effect on SPMD sampling rate for phenanthrene.

Examination of the data for dibenz(a,h)anthracene exposures indicates similar results. After four days, the amounts sequestered by the SPMDs were indistinguishable. The four day K_{LW}s are more variable than phenanthrene's but still within a factor of two between salinity treatments. As observed for phenanthrene, the amount of dibenz(a,h)anthracene sequestered after 28 days was nearly identical for all salinity treatments. However, the K_{LW}s are reversed, i.e. the freshwater K_{LW}s is about 2 fold greater than the ocean water. The higher water concentration of dibenz(a,h)anthracene in the ocean water at 28 days likely results from a rapid initial plating out followed by a slow redissolution throughout the exposure period, as well as disorption from salt particulates. Similar to phenanthrene, there is no evidence for sampling rate dependence on the salinity. Thus, the data from these static exposures suggest that the algorithm developed for fresh water can be used for estuarine and ocean waters as well.

Table 3 gives the physicochemical properties and molecular dimensions of the priority pollutant PAHs. Although the values chosen for log K_{OW} and water solubility were selected as the most "accurate" from numerous literature values, errors of at least two-fold are possible. Also, even though the calculated values of molecular dimensions are reasonably accurate for ideal solutions, they do not reflect the effects of non-ideal solute -

polymer interactions. Therefore, with the exception of molecular weights and ring numbers, the values shown in Table 3 must be viewed as the best available approximations.

Tables 4 A-L, 5 A-L and 6 A-L summarize the results of flow-through diluter exposures of SPMDs to priority pollutant PAHs. In general, SPMDs responded as expected to constant aqueous concentrations of the sixteen priority pollutant PAHs. Concentrations of phenanthrene and larger PAHs, i.e. mw $\geq$ 178, continued to rise in SPMDs throughout the 21-day exposure period. Since the capacity of SPMDs to concentrate an aqueous solute is dependent on the solute's K_{OW} or more precisely its K_{LW} and the four PAHs smaller than phenanthrene have relatively low K_{OW}s ($<2.4 \times 10^4$), some decline in their SPMD R_S values is expected as equilibrium concentrations in the lipid are approached. Figure 1 shows that the R_S for acenaphthylene slowly declines from day 4 through day 21, which is consistent with a first-order rise to equilibrium. Also, Figure 1 shows the expected relatively constant sampling rate throughout the 21-day exposure for PAHs with large log K_{OW}s (i.e. $\geq$ 5.3). The apparent rise in sampling rates from days 4 to 7, and in one case through day 14, is probably due to the lag time required to establish steady state flux across the SPMD membrane. This lag time relates to the membrane penetration time as discussed by Huckins et.al. (2).

Examination of data from all treatments of flow-through SPMD exposures revealed the R_S values for PAHs ranged from a low of less than the MQL ($\simeq$ 0.2 L/d) for naphthalene to a high of 7.5 L/d for pyrene. Earlier unpublished work at the MSC showed that the R_S values for 24 organochlorine pesticides (OCs) in similar tests ranged from 0.2 to 10.6 L/d (not corrected for analytical recovery). Overall, it appears that PAHs may have lower R_S values than those of OCs with similar K_{OW}s.

The K_{OW} or K_{LW} of a compound can be viewed as the driving force for SPMD uptake, whereas compound size and polarity affect resistance to mass transfer. Hildebrand (solubility) parameters are widely used to estimate the solubility of organic solutes in polymers (11) and are a measure of the strength of attraction between molecules of a compound or polymer. Identical or close Hildebrand values of solutes and polymers indicate maximal mutual solubility. Thus for compounds having Hildebrand values close or identical to PE, their uptake should be facile, i.e., minimal resistance to mass transfer. The solubility parameters for different types of polyethylene range from about 7.7 to 8.8 $(cal/cm^3)^{1/2}$. Aroclor® 1248 (a mixture of about 50 polychlorinated biphenyls [PCBs]) has a Hildebrand value of 8.8, whereas naphthalene and anthracene have identical values of 9.9. Although only naphthalene's and anthracene's Hildebrand values

are currently available of the priority pollutant PAHs, their values indicate significantly lower solubility in the PE SPMD membrane than similar PCBs.

Both the effective size (non-ideal interactions included) of a permanent molecule and the size of the available transport corridors or cavities in the polymers are important parameters in the rate of mass transfer through dense or nonporous membranes such as the PE membrane used for SPMDs. Considerable information is available or can be readily derived (molecular modeling) on the dimensions of PAHs at their minimal energy state in an ideal media. Table 3 gives both the derived and literature values of key molecular size descriptors. As can be seen, at least two PAHs have breadths (second largest dimension, cross sectional diameter) that are equivalent to or exceed the theoretical maximum ($\simeq$ 10Å) for transport corridor diameter. Figure 2 shows a plot of molecular breadth versus SPMD R_S values for PAHs. The data are obviously scattered with no apparent correlation of molecular breadth to R_S values. Since many investigators have found positive correlations between breadth and permeability (12), the effects of molecular breadth on SPMD sampling of PAHs may be strictly threshold in nature, i.e. significant impedance to mass transfer is evoked only when the breadth exceeds about 10Å.

Figures 3 and 4 show the potential relationships between SPMD R_S values for PAHs and molecular surface area and volume, respectively. In both plots a parabolic relationship appears to exist. Since the cavities in polyethylene are small and transient in nature, minimal molecular volume should be an important factor in mass transfer rates, as it relates to the number of polymer cavities large enough to accommodate a molecule. Also, as molecular surface area increases stronger matrix interactions are possible especially with the glassy or crystalline regions of the PE (often about 50% of the PE volume). These interactions may be closer to classical adsorption and impedance to mass transfer through the membrane would be due to high activation energies for desorption.

Figure 5 shows the potential relationship between log K_{OW} of PAHs and SPMD R_S values. As observed for molecular volume and surface area plots, a parabolic relationship appears to exist. This type of relationship is also found when gill (fish) uptake efficiencies of recalcitrant organic compounds (those not metabolized) are compared to log K_{OW}s over a wide range of values (13). Plots of log K_{OW} versus log bioconcentration factors (K_B) for a wide range of organic compounds hydrophobicities also result in a commonly observed parabolic curve with log K_B values declining when log K_{OW} exceeds about

6.5 (14). Several reasons are cited for this type of relationship. These include reduced lipid solubility with increased molecular size, extremely low aqueous solubility, and steric factors, i.e. low membrane permeability. All of these factors may affect SPMD sampling rate as well. However, most PAHs remained in the uptake kinetics phase throughout the 21 day SPMD exposure. The direct comparison of kinetics values (SPMD R_S) to equilibrium K_Bs is generally not valid. However, a comparison of a plot of log K_{OW} versus SPMD R_S values to a plot of log K_{OW} and gill extraction efficiency is appropriate and is shown in Figure 6. Although both sets of data appear to describe parabolic relationships, the SPMD data appears to define a much sharper peak. Because the fish data largely represent organochlorine compounds, improved comparisons between the two matrices (i.e., fish gill and polyethylene membrane) await exposures to identical sets of chemicals.

Temperature is known to affect polymer permeability, P, (15). Since SPMD R_S and permeability are very similar (i.e., R_S = PA/l, where A was defined earlier and l is membrane thickness), the temperature of SPMD exposures was expected to affect R_S values of PAHs. Figures 7-10 show plots of water temperature versus R_S values of all sixteen priority pollutant PAHs. In general, R_S values of the PAHs were clearly less affected by

temperature than that observed for OC pesticides. For example, the R_S values of seven PAHs appeared to be unaffected by temperature and any possible difference was well within the variance envelope. Six of these seven PAHs were the smallest sized and lowest molecular weight PAHs tested, i.e., the first six compounds in Table 3. The effects of temperature on SPMD R_S values for PAHs having more than three rings were more complex. Only benz[a]anthracene, benzo[a]pyrene and benzo[g,h,i]perylene appeared to exhibit rising R_S values for the range of temperatures tested. Fluoranthene, pyrene, chrysene and benzo[k]fluoranthene exhibited a substantial rise in R_S values only between 18 and 26^{0}C. Only benzo[k]fluoranthene exhibited a rise from the lowest to maximal R_S value of >50% (i.e. 75%); all others were <50%.

For many organic compounds, the permeability of PE is known to vary with temperature in accordance with an Arrhenius relationship, i.e., plots of 1/K versus ln P, or ln R_S as in our case, would be linear. Figure 11 shows Arrenius plots of acenaphthylene, pyrene, chrysene and benzo[g,h,i]perylene. Three of these four PAHs appeared to have essentially linear plots and the linearity of pyrene was improved. However, as shown earlier, standard plots of temperature versus R_S values of seven of the PAHs tested were linear; thus only in a few cases do variations

in R_S with temperature appear to be in complete accordance with an Arrhenius relationship.

Figures 12-14 show the effects of water concentration on SPMD R_S values for 14 of the 16 PAHs examined. As observed for temperature, possible effects of solute concentration appear to be nonuniform. Because enriched SPMD samples from the 1 ng/L treatment often contained chromatographic interferences, which resulted in NDs and high coefficients of variations, comparison of these values to values from the 10 and 100 ng/L treatments should be viewed with caution. However, the coefficients of variations for all 10 and 100 ng/L R_S values were much smaller. Thus a consistent rise in SPMD R_S values measured at 10 and 100 ng/L appears to indicate a relatively small but significant concentration effects for selected compounds. Any increase in SPMD R_S indicates a deviation from ideal solution behavior in which R_S should be independent of aqueous concentration. When the polymer permeability increases with permanent concentration, it is generally due to solvation of the membrane which increases membrane free volume. This type of interaction can be solute specific and can go through several transitions as concentration rises. Because of this possibility, further investigation at PAH concentrations > 100 ng/L should be considered.

Tables 7 A-F (membrane) and 8 A-F (lipid) summarize the results of freshwater static exposures of SPMDs to a single application of priority pollutant PAHs. The purpose of this study was to estimate values of K_{MW}, K_{LW} and K_{ML} (model parameters used in water concentration estimations) for the priority pollutant PAHs. Based on flow-through exposure (constant concentration) data presented earlier, attainment of steady state concentrations of PAHs in SPMDs may take up to a year for PAHs larger than pyrene. This is due to their moderate to low SPMD R_S values and their generally large steady state K_{LW} (can be viewed as analyte capacity) values. However, using single application static exposures steady state concentrations of PAHs in SPMDs are approached much more rapidly because of the fall of PAH concentrations in the water. Thus, rising concentrations of PAHs in SPMDs are limited or counterbalanced by the rapid depletion of water residues. At some point in time, lipid concentrations reach a maxima and begin to slowly fall.

In these exposures the lipid concentration maxima was observed at 28 days for all PAHs except naphthalene and dibenz[a,h]anthracene. However, the water concentrations of most PAHs continued to fall throughout the 56 day exposures. As a result, a hysteresis effect is possible (15) as SPMDs may not respond instantly (i.e. PAH desorption from SPMDs is slow) to water concentrations below steady state values. Thus, the

possibility exists that PAH K_{LM} can temporarily exceed steady state levels. The rises observed in SPMD concentration factors of most PAHs during the last 28 days of the study was primarily mediated by falling water concentrations (Table 9 A-F).

At the end of these 56 day static exposures, PAH concentration factors in SPMD lipid ranged from a low of 2,200 (naphthalene) to a high of 680,000 (benzo[b]fluoranthene). Lipid concentration factors of PAHs (28 and 56 day samples) appear to rise with K_{OW} values, up to benzo[b]fluoranthene (log K_{OW} = 5.78), and then decline as molecular size and K_{OW} continues to increase. It appears that a parabolic relationship exists for SPMD lipid concentration factors and log K_{OW}s (Figure 15) similar to plots of SPMD R_S values and log K_{OW}s presented earlier.

As expected K_{MW} or membrane concentration factors were always less than K_{LW} values. At 56 days they ranged from a low of 304 (naphthalene) to a high of 280,000 (benzo[b]fluoranthene) which is probably well above the actual steady state value. The distribution of PAHs between the SPMD membrane and lipid K_{ML} at the 28 day lipid maxima ranged from a low of 0.08 for naphthalene to a high of 0.56 for ideno[1,2,3,c,d]pyrene. The magnitude of PAH K_{ML} values appeared to rise in concert with increasing

molecular size. For PAHs with molecular weights greater than benz[a]anthracene, reduced lipid solubility may play a role in the rise of their K_{ML}s. However, other factors affecting K_{ML}s such as greater adsorptive interactions with the polyethylene membrane chains in rubbery and in particular crystalline regions of the polymer, which are known to increase with molecular surface area.

CONCLUSIONS

SPMD theory was in accordance with the PAH data generated in these studies. In particular, SPMD sampling rates rose with PAH K_{OW} values until molecular size impeded or reduced membrane permeability and lipid solubility. This phenomenon is similar to that observed for the bioconcentration of recalcitrant contaminants having very large K_{OW} values. Thus, the SPMD appears to mimic the bioconcentration process but can be used for readily metabolized compounds such as PAHs as well. Laboratory calibration data was generated in this project that will ultimately permit accurate SPMD based estimations of PAH concentrations in diverse aqueous environments.

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TABLES

TABLE 1: STATIC EXPOSURES (SINGLE APPLICATION AT 18°C) OF SPMDs TO MODEL ^{14}C-PAHs IN FRESH AND SALINE WATERS

		Mean (N=3) of Applied PAHs Sequestered in SPMDs (Lipid and Membrane)								
		FRESH (WELL) WATER			ESTUARINE WATER			OCEAN WATER		
Analyte	Study Day	CF in Lipid	% Sequestered in SPMDs	C.V.	CF in Lipid	% Sequestered in SPMDs	C.V.	CF in Lipid	% Sequestered in SPMDs	C.V.
Phenanthrene										
	4	11,600	81	3	11,600	80	3	11,000	81	4
	7	28,300	85	3	35,700	84	12	30,300	87	2
	14	32,300	87	2	36,800	91	2	54,200	95	1
	28	58,000	87	3	76,700	84	0[1]	99,000	87	1
Dibenz(a,h)anthracene										
	4	1,600	60	4	1,100	52	2	2,800	47	5
	7	5,600	74	9	3,200	70	6	5,600	55	3
	14	16,700	85	3	10,000	70	12	8,900	62	7
	28	48,400	92	3	35,000	92	3	23,000	90	4

[1]Only two (N=2) replicates, one sample lost

TABLE 2-A: STATIC EXPOSURES (SINGLE APPLICATION AT 18°C) OF SPMDs TO MODEL ^{14}C-PAHs IN FRESH AND SALINE WATERS

Analyte	Study Day	Amount Spiked in Water DPM	AMOUNT SEQUESTERED IN SPMDs						
			FRESH (WELL) WATER						
			Rep 1 DPM	Rep 2 DPM	Rep 3 DPM	Mean	S.D.	C.V.	% Recovered in SPMDs
Phenanthrene	4	131,942	110,246	103,319	108,084	107,216	3,544	3	81
	7	132,164	116,213	109,606	111,725	112,515	3,374	3	85
	14	131,616	114,340	111,963	117,358	114,554	2,704	2	87
	28	131,942	117,641	116,494	111,870	115,335	3,055	3	87
Dibenz(a,h)athracene	4	10,760	6,687	6,313	6,221	6,407	247	4	60
	7	9,859	7,474	6,504	7,804	7,261	676	9	74
	14	10,172	8,947	8,535	8,400	8,627	285	3	85
	28	10,172	9,556	9,461	9,083	9,367	250	3	92

TABLE 2-B: STATIC EXPOSURES (SINGLE APPLICATION AT 18°C) OF SPMDs TO MODEL ^{14}C-PAHs IN FRESH AND SALINE WATERS

		AMOUNT SEQUESTERED IN SPMDs						
		ESTUARINE WATER						
Analyte	Study Day	Rep 1 DPM	Rep 2 DPM	Rep 3 DPM	Mean	S.D.	C.V.	% Recovered in SPMDs
Phenanthrene	4	103,006	104,655	107,417	105,026	2,229	2	80
	7	96,285	113,448	121,568	110,434	12,908	12	84
	14	121,636	118,146	120,917	120,233	1,843	2	91
	28	NA	110,503	110,443	110,473	42	0	84
Dibenz(a,h)athracene	4	5,702	5,468	5,504	5,558	126	2	52
	7	7,234	6,467	6,999	6,900	393	6	70
	14	6,621	8,157	6,666	7,148	874	12	70
	28	9,586	9,470	9,071	9,376	270	3	92

TABLE 2-C: STATIC EXPOSURES (SINGLE APPLICATION AT 18°C) OF SPMDs TO MODEL ^{14}C-PAHs IN FRESH AND SALINE WATERS

		AMOUNT SEQUESTERD IN SPMDs						
		OCEAN WATER						
Analyte	Study Day	Rep 1 DPM	Rep 2 DPM	Rep 3 DPM	Mean	S.D.	C.V.	% Recovered in SPMDs
Phenanthrene	4	111,920	103,709	105,315	106,981	4,352	4	81
	7	116,901	111,535	115,174	114,537	2,739	2	87
	14	124,175	124,611	125,676	124,821	722	1	95
	28	114,919	115,434	112,553	114,302	1,536	1	87
Dibenz(a,h)athracene	4	4,935	5,357	4,837	5,043	276	5	47
	7	5,388	5,352	5,616	5,452	143	3	55
	14	6,735	6,309	5,816	6,287	460	7	62
	28	9,025	8,956	9,579	9,187	342	4	90

Table 3 Molecular properties of priority pollutant PAHs

Compound	MW[1]	No. of rings[2]	Minimal Box Dimension(Å) Estimates[3] Length (L)	Breadth (B)	Depth (D)	L/B ratio	TSA[4] (Å^2)	Molecular[4] Volume (Å^3)	Water Solubility[5] mg/L	log K_{ow}[5]
Naphthalene	128.2	2A	8.9	7.2	3.1	1.2	155.8	126.9	30.2	3.45
Acenaphthylene	152.2	2A1C	8.8	8.4	3.1	1.0	182.0	-	3.93	4.08
Acenaphthene	154.2	2A1C	8.8	8.1	3.2	1.1	180.1	148.8	3.93	4.22
Fluorene	166.2	2A1C	11.1	7.2	3.1	1.5	194.0	160.4	1.90	4.38
Phenanthrene	178.2	3A	11.5	7.7	3.1	1.5	199.4	169.5	1.18	4.46
Anthracene	178.2	3A	11.7	7.2	3.1	1.6	203.5	170.3	0.076	4.54
Fluoranthene	202.3	3A1C	10.7	9.0	3.1	1.2	218.6	187.7	0.260	5.20
Pyrene	202.3	4A	11.4	9.5	3.1	1.2	213.5	186.0	0.135	5.30
Benz[a] anthracene	228.3	4A	13.7	9.4	3.1	1.4	244.3	212.9	0.011	5.91
Chrysene	228.3	4A	13.6	7.7	4.4	1.8	240.1	212.2	0.0019	5.61
Benzo[b] fluoranthene	252.3	4A1C	13.6	10.4 (9.3)[6]	4.5	1.3	260.8	230.3	0.0140	5.78
Benzo[k] fluoranthene	252.3	4A1C	13.3	9.1	3.1	1.5	265.0	231.1	0.0080	6.20
Benzo[a]pyrene	252.3	5A	13.6	8.9	3.1	1.5	255.6	228.6	0.0038	6.35
Dibenz[a,h] anthracene	278.4	5A	15.6	9.3	3.1	1.7	286.5	244.3	0.0005	6.75
Indeno[1,2,3-cd] pyrene	267.0	5A1C	13.2	10.0	3.1	1.3			0.0005	6.51
Benzo[g,h,i] perylene	276.3	5A1C	11.5	10.2	3.1	1.1	266.9	244.3	0.0003	6.90

[1] Molecular weight

[2] A = aromatic, C = nonaromatic

[3] Calculated for minimal molecular energy configuration, using Alchemy III and convention described by Sander and Wise (ref)

[4] Total surface area of molecule, data from Pearlman et.al. (1984)

[5] Preferred or selected values from Mackay et.al. "Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals," Vol II (1992)

[6] Alternative breadth value; exact value uncertain because of problems associated with shape and deviation from planarity

TABLE 4 A. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(Low Water Concentration, 1 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	3	ND	ND	3	---	---	1	1.1	916	1.3
acenaphthene	7	2	ND	4	3	69	2	1.5	1,102	1.5
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	4	3	4	3	0	8	1	1.1	1,271	1.7
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	2	12	11	8	5	56	3	1.3	2,595	3.5
benz[a]anthracene	5	3	2	3	1	33	1	1.1	1,173	1.6
chrysene	2	2	2	2	0	14	1	1.1	611	0.8
benzo[b]fluoranthene	4	4	2	3	1	22	1	1.4	950	1.3
benzo[k]fluoranthene	6	5	ND	5	---	---	2	1.3	1,628	2.2
benzo[a]pyrene	2	6	4	4	2	41	2	1.1	1,405	1.9
indeno[123cd]pyrene	3	4	ND	3	---	---	1	1.1	1,228	1.7
dibenz[ah]anthracene	2	4	ND	3	---	---	1	1.1	1,063	1.5
benzo[ghi]perylene	3	3	3	3	0	3	1	1.9	665	0.9

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 B. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(Low Water Concentration, 1 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
acenaphthene	2	3	2	2	0	20	1	1.5	618	0.5
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	3	3	2	2	0	14	1	1.3	734	0.6
benz[a]anthracene	8	9	5	7	2	24	3	1.1	2,578	2.0
chrysene	4	4	3	4	1	21	1	1.1	1,356	1.1
benzo[b]fluoranthene	7	8	6	7	1	15	3	1.4	1,978	1.5
benzo[k]fluoranthene	8	8	6	7	1	15	3	1.3	2,223	1.7
benzo[a]pyrene	10	11	7	9	2	21	4	1.1	3,470	2.7
indeno[123cd]pyrene	5	5	2	4	1	34	2	1.1	1,478	1.2
dibenz[ah]anthracene	4	4	2	3	1	23	1	1.1	1,222	1.0
benzo[ghi]perylene	5	5	3	4	1	16	2	1.9	884	0.7

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 C. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(Low Water Concentration, 1 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	2	2	2	2	0	6	1	1.1	611	0.2
acenaphthene	5	4	4	4	1	16	2	1.5	1,183	0.5
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	18	19	17	18	1	3	7	1.1	6,549	2.6
chrysene	11	12	10	11	1	7	5	1.1	4,106	1.6
benzo[b]fluoranthene	71	17	16	35	26	74	14	1.4	9,946	3.9
benzo[k]fluoranthene	37	19	15	23	10	41	9	1.3	7,206	2.8
benzo[a]pyrene	10	15	13	13	2	18	5	1.1	4,680	1.8
indeno[123cd]pyrene	10	15	16	13	2	17	5	1.1	4,936	1.9
dibenz[ah]anthracene	22	9	8	13	6	49	5	1.1	4,753	1.9
benzo[ghi]perylene	ND	110	ND	110	---	---	44	1.9	23,323	9.1

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 D. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(Low Water Concentration, 1 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Average	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	2	2	2	2	0	4	1	1.1	733	0.2
acenaphthene	5	5	5	5	0	5	2	1.5	1,335	0.3
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	19	ND	ND	19	---	---	8	1.1	7,111	1.8
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	54	48	46	49	3	7	20	1.3	15,261	4.0
benz[a]anthracene	30	28	26	28	2	6	11	1.1	10,215	2.7
chrysene	18	15	13	15	2	12	6	1.1	5,523	1.4
benzo[b]fluoranthene	35	43	22	33	9	26	13	1.4	9,514	2.5
benzo[k]fluoranthene	28	24	19	24	4	16	10	1.3	7,341	1.9
benzo[a]pyrene	26	18	16	20	4	22	8	1.1	7,392	1.9
indeno[123cd]pyrene	22	20	17	19	2	10	8	1.1	7,111	1.8
dibenz[ah]anthracene	17	12	16	15	2	15	6	1.1	5,486	1.4
benzo[ghi]perylene	ND	ND	ND	ND	---	---	ND	1.9	ND	ND

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 E. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C (Medium Water Concentration, 10 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	9	10	10	10	0	4	4	9	456	0.6
acenaphthene	17	18	20	18	1	6	7	11	641	0.9
fluorene	24	25	26	25	1	3	10	8	1,309	1.8
phenanthrene	43	34	34	37	4	11	15	8	1,925	2.6
anthracene	36	31	32	33	2	7	13	10	1,362	1.9
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	56	55	62	58	3	5	23	8	3,018	4.1
benz[a]anthracene	44	40	42	42	1	3	17	10	1,623	2.2
chrysene	44	43	47	45	2	4	18	11	1,579	2.2
benzo[b]fluoranthene	37	36	38	37	1	2	15	12	1,221	1.7
benzo[k]fluoranthene	35	38	38	37	1	3	15	12	1,218	1.7
benzo[a]pyrene	38	41	41	40	2	4	16	12	1,382	1.9
indeno[123cd]pyrene	25	22	26	24	2	8	10	11	929	1.3
dibenz[ah]anthracene	19	15	16	17	2	10	7	11	625	0.9
benzo[ghi]perylene	29	31	30	30	1	2	12	15	825	1.1

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 F. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(Medium Water Concentration, 10 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	13	13	---	---	5	12	448	0.3
acenaphthylene	17	20	17	18	1	8	7	9	844	0.7
acenaphthene	31	34	37	34	2	7	14	11	1,201	0.9
fluorene	39	43	48	43	4	9	17	8	2,290	1.8
phenanthrene	50	62	72	61	9	14	25	8	3,217	2.5
anthracene	52	61	67	60	6	11	24	10	2,490	1.9
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	87	92	94	91	3	3	37	8	4,769	3.7
benz[a]anthracene	71	79	81	77	4	6	31	10	2,984	2.3
chrysene	75	89	91	85	7	9	34	11	2,998	2.3
benzo[b]fluoranthene	75	70	73	73	2	3	29	12	2,400	1.9
benzo[k]fluoranthene	60	66	71	66	4	7	27	12	2,155	1.7
benzo[a]pyrene	73	71	73	73	1	1	29	12	2,521	2.0
indeno[123cd]pyrene	44	59	58	54	7	13	22	11	2,060	1.6
dibenz[ah]anthracene	32	38	48	39	7	17	16	11	1,459	1.1
benzo[ghi]perylene	47	54	54	52	3	7	21	15	1,433	1.1

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 G. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(Medium Water Concentration, 10 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	17	23	17	19	3	16	8	9	865	0.3
acenaphthene	38	48	35	41	6	14	16	11	1,437	0.6
fluorene	51	64	49	55	7	12	22	8	2,915	1.1
phenanthrene	104	105	76	95	13	14	38	8	4,956	1.9
anthracene	95	107	75	93	13	14	37	10	3,849	1.5
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	142	149	119	137	13	9	55	8	7,167	2.8
benz[a]anthracene	153	157	135	148	10	6	60	10	5,738	2.2
chrysene	175	170	147	164	12	8	66	11	5,797	2.3
benzo[b]fluoranthene	139	159	127	142	13	9	57	12	4,687	1.8
benzo[k]fluoranthene	147	126	126	133	10	7	54	12	4,352	1.7
benzo[a]pyrene	137	145	129	137	6	5	55	12	4,754	1.9
indeno[123cd]pyrene	115	123	90	109	14	13	44	11	4,190	1.6
dibenz[ah]anthracene	72	59	68	66	5	8	27	11	2,455	1.0
benzo[ghi]perylene	99	106	83	96	9	10	39	15	2,647	1.0

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 H. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(Medium Water Concentration, 10 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Average	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	20	29	19	23	4	18	9	9	1,058	0.3
acenaphthene	46	70	41	52	13	24	21	11	1,848	0.5
fluorene	71	59	62	64	5	8	26	8	3,403	0.9
phenanthrene	152	211	129	164	34	21	66	8	8,579	2.2
anthracene	143	189	135	155	24	15	63	10	6,463	1.7
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	271	376	248	298	56	19	120	8	15,611	4.1
benz[a]anthracene	221	338	204	254	60	23	103	10	9,865	2.6
chrysene	232	368	236	279	63	23	112	11	9,851	2.6
benzo[b]fluoranthene	201	302	180	228	53	23	92	12	7,522	2.0
benzo[k]fluoranthene	198	301	198	232	48	21	94	12	7,615	2.0
benzo[a]pyrene	199	312	185	232	57	24	94	12	8,061	2.1
indeno[123cd]pyrene	151	258	129	179	56	31	72	11	6,888	1.8
dibenz[ah]anthracene	124	341	67	177	118	66	71	11	6,555	1.7
benzo[ghi]perylene	146	215	130	164	37	23	66	15	4,521	1.2

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 I. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(High Water Concentration, 100 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	45	66	60	57	9	15	23	79	292	0.4
acenaphthylene	208	176	181	188	14	8	76	103	738	1.0
acenaphthene	256	226	241	241	12	5	97	103	943	1.3
fluorene	314	286	278	293	16	5	118	89	1,323	1.8
phenanthrene	441	349	379	390	39	10	157	90	1,751	2.4
anthracene	413	330	354	366	35	9	147	113	1,308	1.8
fluoranthene	ND [4]	ND	ND	ND	---	---	ND	94	ND	ND
pyrene	551	373	449	457	73	16	184	85	2,173	3.0
benz[a]anthracene	452	338	367	386	49	13	156	104	1,501	2.0
chrysene	398	314	333	348	36	10	140	103	1,358	1.9
benzo[b]fluoranthene	432	313	338	361	51	14	146	123	1,185	1.6
benzo[k]fluoranthene	377	302	312	330	33	10	133	118	1,126	1.5
benzo[a]pyrene	379	321	370	356	26	7	144	99	1,452	2.0
indeno[123cd]pyrene	345	248	244	279	47	17	112	93	1,204	1.6
dibenz[ah]anthracene	248	171	130	183	49	27	74	108	680	0.9
benzo[ghi]perylene	372	265	283	306	47	15	124	126	978	1.3

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 J. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(High Water Concentration, 100 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	52	98	61	70	20	28	28	79	359	0.3
acenaphthylene	267	242	245	251	11	4	101	103	985	0.8
acenaphthene	368	342	343	351	12	3	141	103	1,372	1.1
fluorene	458	402	459	440	27	6	177	89	1,987	1.5
phenanthrene	663	604	654	640	26	4	258	90	2,879	2.2
anthracene	627	532	601	587	40	7	237	113	2,099	1.6
fluoranthene	697	565	667	643	57	9	259	94	2,747	2.1
pyrene	820	712	804	779	48	6	314	85	3,699	2.9
benz[a]anthracene	681	575	702	653	55	9	263	104	2,538	2.0
chrysene	716	552	763	677	91	13	273	103	2,640	2.1
benzo[b]fluoranthene	658	541	648	615	53	9	248	123	2,019	1.6
benzo[k]fluoranthene	594	491	602	562	51	9	227	118	1,916	1.5
benzo[a]pyrene	601	543	597	580	26	5	234	99	2,364	1.8
indeno[123cd]pyrene	525	413	475	471	46	10	190	93	2,032	1.6
dibenz[ah]anthracene	456	339	380	392	48	12	158	108	1,456	1.1
benzo[ghi]perylene	472	446	447	455	12	3	183	126	1,451	1.1

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).

TABLE 4 K. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(High Water Concentration, 100 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	66	54	60	60	5	9	24	79	308	0.1
acenaphthylene	385	338	351	358	20	5	144	103	1,404	0.5
acenaphthene	647	566	581	598	35	6	241	103	2,339	0.9
fluorene	905	733	797	811	71	9	327	89	3,668	1.4
phenanthrene	1,437	1,249	1,349	1,345	77	6	542	90	6,044	2.4
anthracene	1,364	1,321	1,280	1,322	34	3	533	113	4,729	1.8
fluoranthene	ND [4]	1,707	ND	1,707	---	---	688	94	7,293	2.8
pyrene	2,135	1,712	1,747	1,865	192	10	752	85	8,857	3.5
benz[a]anthracene	1,812	1,543	1,461	1,605	150	9	647	104	6,242	2.4
chrysene	1,872	1,570	1,464	1,635	173	11	659	103	6,376	2.5
benzo[b]fluoranthene	1,749	1,458	1,370	1,526	162	11	615	123	5,005	2.0
benzo[k]fluoranthene	1,721	1,427	1,364	1,504	156	10	606	118	5,125	2.0
benzo[a]pyrene	1,583	1,330	1,259	1,391	139	10	561	99	5,664	2.2
indeno[123cd]pyrene	1,215	1,075	942	1,077	112	10	434	93	4,651	1.8
dibenz[ah]anthracene	842	871	678	797	85	11	321	108	2,965	1.2
benzo[ghi]perylene	1,136	1,059	896	1,030	100	10	415	126	3,286	1.3

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 4 L. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 10 °C
(High Water Concentration, 100 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Average	Std Dev.	C.V.				
naphthalene	93	80	---	87	---	---	35	79	443	0.1
acenaphthylene	515	437	---	476	---	---	192	103	1,866	0.5
acenaphthene	818	688	---	753	---	---	304	103	2,946	0.8
fluorene	1,204	1,042	---	1,123	---	---	453	89	5,076	1.3
phenanthrene	1,981	1,773	---	1,877	---	---	757	90	8,435	2.2
anthracene	1,907	1,759	---	1,833	---	---	739	113	6,559	1.7
fluoranthene	ND [4]	ND	---	ND	---	---	ND	94	ND	ND
pyrene	3,562	2,548	---	3,055	---	---	1,232	85	14,508	3.8
benz[a]anthracene	2,611	2,137	---	2,374	---	---	957	104	9,230	2.4
chrysene	2,638	2,368	---	2,503	---	---	1,009	103	9,760	2.5
benzo[b]fluoranthene	2,477	1,928	---	2,202	---	---	888	123	7,226	1.9
benzo[k]fluoranthene	2,258	1,766	---	2,012	---	---	811	118	6,858	1.8
benzo[a]pyrene	2,252	1,746	---	1,999	---	---	806	99	8,141	2.1
indeno[123cd]pyrene	1,744	1,288	---	1,516	---	---	611	93	6,545	1.7
dibenz[ah]anthracene	1,246	947	---	1,096	---	---	442	108	4,078	1.1
benzo[ghi]perylene	1,622	1,324	---	1,473	---	---	594	126	4,699	1.2

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 A. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C (Low Water Concentration, 1 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
acenaphthene	6	5	5	5	1	13	2	1.5	1,416	1.9
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	7	6	6	6	0	5	3	1.1	2,370	3.2
chrysene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
benzo[b]fluoranthene	5	4	6	5	1	17	2	1.4	1,363	1.9
benzo[k]fluoranthene	2	4	2	2	1	33	1	1.3	765	1.0
benzo[a]pyrene	4	4	3	4	0	12	2	1.1	1,369	1.9
indeno[123cd]pyrene	4	3	3	3	0	9	1	1.1	1,185	1.6
dibenz[ah]anthracene	4	2	2	3	1	25	1	1.1	978	1.3
benzo[ghi]perylene	2	ND	2	2	---	---	1	1.9	478	0.7

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 B. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(Low Water Concentration, 1 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	4	2	2	3	1	41	1	1.1	1,014	0.8
acenaphthene	ND	ND	ND	ND	---	---	ND	1.5	ND	ND
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	ND	158	ND	158	---	---	64	1.3	49,132	38.3
benz[a]anthracene	10	9	11	10	1	10	4	1.1	3,641	2.8
chrysene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
benzo[b]fluoranthene	9	8	11	9	1	15	4	1.4	2,659	2.1
benzo[k]fluoranthene	7	9	13	10	2	22	4	1.3	3,009	2.3
benzo[a]pyrene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
indeno[123cd]pyrene	8	2	6	5	3	48	2	1.1	1,967	1.5
dibenz[ah]anthracene	9	3	6	6	2	37	2	1.1	2,175	1.7
benzo[ghi]perylene	11	6	6	7	2	32	3	1.9	1,542	1.2

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 C. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(Low Water Concentration, 1 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	47	ND [4]	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	ND	3	7	5	---	---	2	1.1	1,778	0.7
acenaphthene	ND	ND	ND	ND	---	---	ND	1.5	ND	ND
fluorene	30	ND	ND	30	---	---	12	0.5	24,032	9.4
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	15	24	16	18	4	22	7	1.1	6,623	2.6
chrysene	4,647	24	ND	24	---	---	9	1.1	8,614	3.4
benzo[b]fluoranthene	14	18	14	16	2	13	6	1.4	4,474	1.7
benzo[k]fluoranthene	15	18	12	15	3	17	6	1.3	4,694	1.8
benzo[a]pyrene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
indeno[123cd]pyrene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
dibenz[ah]anthracene	8	10	8	9	1	9	4	1.1	3,201	1.2
benzo[ghi]perylene	395	8	7	8	---	---	3	1.9	1,613	0.6

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 D. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(Low Water Concentration, 1 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
acenaphthene	ND	ND	ND	ND	---	---	ND	1.5	ND	ND
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	120	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	31	34	26	30	3	10	12	1.1	11,083	2.9
chrysene	27	28	22	26	2	9	10	1.1	9,372	2.4
benzo[b]flnoranthene	25	30	25	27	2	8	11	1.4	7,690	2.0
benzo[k]fluoranthene	20	28	25	24	3	14	10	1.3	7,517	2.0
beuzo[a]pyrene	19	25	23	22	2	10	9	1.1	8,187	2.1
indeno[123cd]pyrene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
dibenz[ah]anthracene	17	15	16	16	0	3	6	1.1	5,890	1.5
benzo[ghi]perylene	7	11	9	9	1	15	4	1.9	1,924	0.5

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 E. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C (Medium Water Concentration, 10 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	ND	ND	ND	ND	---	---	ND	9	ND	ND
acenaphthene	19	13	18	16	3	16	7	11	575	0.8
fluorene	17	20	15	17	2	10	7	8	911	1.2
phenanthrene	ND	ND	ND	ND	---	---	ND	8	ND	ND
anthracene	20	11	17	16	4	23	6	10	665	0.9
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	50	23	37	37	11	29	15	8	1,929	2.6
benz[a]anthracene	70	39	60	56	13	23	23	10	2,185	3.0
chrysene	62	32	36	43	13	30	17	11	1,530	2.1
benzo[b]fluoranthene	57	31	43	43	11	25	18	12	1,434	2.0
benzo[k]fluoranthene	48	29	32	36	9	24	15	12	1,187	1.6
benzo[a]pyrene	43	27	42	37	7	19	15	12	1,302	1.8
indeno[123cd]pyrene	24	16	23	21	4	17	8	11	804	1.1
dibenz[ah]anthracene	23	18	12	18	5	26	7	11	655	0.9
benzo[ghi]perylene	29	17	21	22	5	22	9	15	609	0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 F. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(Medium Water Concentration, 10 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	15	18	26	20	5	23	8	9	921	0.7
acenaphthene	27	27	25	26	1	4	11	11	931	0.7
fluorene	ND	ND	ND	ND	---	---	ND	8	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	8	ND	ND
anthracene	40	59	57	52	9	16	21	10	2,160	1.7
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	ND	87	99	93	6	7	37	8	4,857	3.8
benz[a]anthracene	127	134	133	131	3	3	53	10	5,089	4.0
chrysene	101	136	119	118	14	12	48	11	4,183	3.3
benzo[b]fluoranthene	83	105	95	94	9	10	38	12	3,112	2.4
benzo[k]fluoranthene	79	89	92	87	5	6	35	12	2,838	2.2
benzo[a]pyrene	91	76	110	92	14	15	37	12	3,213	2.5
indeno[123cd]pyrene	43	49	53	48	4	8	19	11	1,856	1.4
dibenz[ah]anthracene	46	49	56	50	4	9	20	11	1,864	1.5
benzo[ghi]perylene	44	51	72	55	12	21	22	15	1,528	1.2

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 G. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(Medium Water Concentration, 10 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	26	23	36	28	6	20	11	9	1,307	0.5
acenaphthene	45	43	41	43	2	4	17	11	1,524	0.6
fluorene	81	87	83	84	2	3	34	8	4,435	1.7
phenanthrene	124	125	128	126	2	1	51	8	6,572	2.6
anthracene	108	97	98	101	5	5	41	10	4,197	1.6
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	153	155	138	149	7	5	60	8	7,790	3.0
benz[a]anthracene	198	183	174	185	10	5	75	10	7,164	2.8
chrysene	239	177	204	207	25	12	83	11	7,309	2.8
benzo[b]fluoranthene	164	154	133	150	13	9	61	12	4,963	1.9
benzo[k]fluoranthene	163	145	138	148	10	7	60	12	4,864	1.9
benzo[a]pyrene	159	163	140	154	10	6	62	12	5,343	2.1
indeno[123cd]pyrene	112	112	61	95	24	25	38	11	3,650	1.4
dibenz[ah]anthracene	97	119	55	90	26	29	36	11	3,339	1.3
benzo[ghi]perylene	123	136	76	112	26	23	45	15	3,083	1.2

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 H. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(Medium Water Concentration, 10 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	44	34	44	41	5	11	16	9	1,877	0.5
acenaphthene	50	44	51	48	3	7	19	11	1,697	0.4
fluorene	110	88	99	99	9	9	40	8	5,251	1.4
phenanthrene	ND	ND	ND	ND	---	---	ND	8	ND	ND
anthracene	150	132	156	146	10	7	59	10	6,062	1.6
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	ND	ND	ND	ND	---	---	ND	8	ND	ND
benz[a]anthracene	256	233	244	244	10	4	99	10	9,473	2.5
chrysene	341	271	326	313	30	10	126	11	11,060	2.9
benzo[b]fluoranthene	209	197	209	205	6	3	83	12	6,767	1.8
benzo[k]fluoranthene	239	219	231	230	8	4	93	12	7,528	2.0
benzo[a]pyrene	238	222	234	231	7	3	93	12	8,037	2.1
indeno[123cd]pyrene	160	138	150	149	9	6	60	11	5,733	1.5
dibenz[ah]anthracene	165	141	151	152	10	6	61	11	5,629	1.5
benzo[ghi]perylene	151	142	146	146	4	3	59	15	4,035	1.0

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 I. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(High Water Concentration, 100 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	33	42	41	39	4	10	16	79	198	0.3
acenaphthylene	174	168	180	174	5	3	70	103	682	0.9
acenaphthene	243	217	232	231	10	5	93	103	902	1.2
fluorene	226	194	203	207	13	6	84	89	938	1.3
phenanthrene	478	347	385	403	55	14	163	90	1,813	2.5
anthracene	454	296	411	387	67	17	156	113	1,385	1.9
fluoranthene	ND [4]	ND	ND	ND	---	---	ND	94	ND	ND
pyrene	466	464	497	476	15	3	192	85	2,260	3.1
benz[a]anthracene	451	331	356	379	51	14	153	104	1,474	2.0
chrysene	537	433	490	487	43	9	196	103	1,897	2.6
benzo[b]fluoranthene	401	287	304	330	50	15	133	123	1,084	1.5
benzo[k]fluoranthene	480	444	426	450	23	5	181	118	1,534	2.1
benzo[a]pyrene	413	357	356	375	26	7	151	99	1,528	2.1
indeno[123cd]pyrene	300	230	240	257	31	12	103	93	1,108	1.5
dibenz[ah]anthracene	318	224	253	265	39	15	107	108	986	1.3
benzo[ghi]perylene	217	182	192	197	15	7	80	126	630	0.9

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 J. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(High Water Concentration, 100 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	37	31	46	38	6	17	15	79	193	0.2
acenaphthylene	264	231	269	255	17	7	103	103	999	0.8
acenaphthene	401	312	382	365	38	11	147	103	1,428	1.1
fluorene	410	267	386	354	63	18	143	89	1,602	1.2
phenanthrene	851	608	798	752	104	14	303	90	3,382	2.6
anthracene	892	550	823	755	147	20	304	113	2,702	2.1
fluoranthene	ND [4]	ND	ND	ND	---	---	ND	94	ND	ND
pyrene	976	748	926	883	98	11	356	85	4,195	3.3
benz[a]anthracene	936	658	911	835	126	15	337	104	3,247	2.5
chrysene	972	796	1,032	933	100	11	376	103	3,639	2.8
benzo[b]fluoranthene	910	623	846	793	123	16	320	123	2,602	2.0
benzo[k]fluoranthene	1,047	709	982	913	146	16	368	118	3,111	2.4
benzo[a]pyrene	885	606	834	775	121	16	312	99	3,156	2.5
indeno[123cd]pyrene	722	439	605	588	116	20	237	93	2,540	2.0
dibenz[ah]anthracene	642	386	529	519	105	20	209	108	1,930	1.5
benzo[ghi]perylene	652	310	395	452	145	32	182	126	1,442	1.1

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 K. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(High Water Concentration, 100 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Mean Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	PAHs Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	29	38	80	49	22	46	20	79	251	0.1
acenaphthylene	360	328	416	368	36	10	148	103	1,443	0.6
acenaphthene	574	505	580	553	34	6	223	103	2,163	0.8
fluorene	698	673	743	704	29	4	284	89	3,184	1.2
phenanthrene	1,479	1,204	1,459	1,381	125	9	557	90	6,206	2.4
anthracene	1,531	1,342	1,455	1,443	78	5	582	113	5,162	2.0
fluoranthene	1,445	ND [4]	1,774	1,610	---	---	649	94	6,876	2.7
pyrene	1,925	1,448	1,789	1,721	200	12	694	85	8,171	3.2
benz[a]anthracene	1,971	1,563	1,755	1,763	166	9	711	104	6,855	2.7
chrysene	2,054	1,718	1,915	1,896	138	7	764	103	7,393	2.9
benzo[b]fluoranthene	1,792	1,496	1,681	1,656	122	7	668	123	5,434	2.1
benzo[k]fluoranthene	1,831	1,596	1,757	1,728	98	6	697	118	5,890	2.3
benzo[a]pyrene	1,575	1,436	1,619	1,544	78	5	622	99	6,287	2.5
indeno[123cd]pyrene	1,210	1,253	1,146	1,203	44	4	485	93	5,193	2.0
dibenz[ah]anthracene	1,072	1,052	1,147	1,090	41	4	439	108	4,054	1.6
benzo[ghi]perylene	1,156	1,133	1,297	1,195	73	6	482	126	3,813	1.5

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 5 L. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 18 °C
(High Water Concentration, 100 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	56	76	59	63	9	14	26	79	325	0.1
acenaphthylene	403	425	350	393	31	8	158	103	1,541	0.4
acenaphthene	615	662	525	600	57	9	242	103	2,348	0.6
fluorene	952	937	792	894	72	8	360	89	4,041	1.0
phenanthrene	2,017	2,012	2,186	2,071	81	4	835	90	9,312	2.4
anthracene	1,908	1,683	1,405	1,665	206	12	672	113	5,959	1.5
fluoranthene	ND [4]	ND	ND	ND	---	---	ND	94	ND	ND
pyrene	2,665	2,336	2,374	2,458	147	6	991	85	11,675	3.0
benz[a]anthracene	2,833	2,417	2,455	2,568	188	7	1,036	104	9,986	2.6
chrysene	3,112	2,550	2,622	2,761	249	9	1,113	103	10,768	2.8
benzo[b]fluoranthene	2,875	2,313	2,400	2,529	247	10	1,020	123	8,298	2.2
benzo[k]fluoranthene	2,917	2,402	2,446	2,588	233	9	1,044	118	8,822	2.3
benzo[a]pyrene	2,699	2,143	2,249	2,364	241	10	953	99	9,627	2.5
indeno[123cd]pyrene	2,231	1,693	1,711	1,878	249	13	757	93	8,107	2.1
dibenz[ah]anthracene	1,795	1,369	1,316	1,493	214	14	602	108	5,554	1.4
benzo[ghi]perylene	1,767	1,536	1,297	1,533	192	13	618	126	4,890	1.3

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 A. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(Low Water Concentration, 1 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	ND	ND	4	4	---	---	2	1.1	1,430	2.0
acenaphthene	ND	4	4	4	---	---	2	1.5	1,169	1.6
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	8	8	10	9	1	11	4	1.1	3,226	4.4
chrysene	10	10	13	11	1	13	4	1.1	3,996	5.5
benzo[b]fluoranthene	8	7	10	8	1	14	3	1.4	2,419	3.3
benzo[k]fluoranthene	12	10	15	12	2	16	5	1.3	3,856	5.3
benzo[a]pyrene	8	7	13	10	3	28	4	1.1	3,482	4.8
indeno[123cd]pyrene	4	4	8	5	2	41	2	1.1	1,955	2.7
dibenz[ah]anthracene	4	2	8	5	2	52	2	1.1	1,735	2.4
benzo[ghi]perylene	4	5	6	5	1	12	2	1.9	1,061	1.4

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 B. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(Low Water Concentration, 1 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	ND	6	3	4	---	---	2	1.1	1,558	1.2
acenaphthene	ND	ND	ND	ND	---	---	ND	1.5	ND	ND
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	7	6	5	6	1	10	2	1.1	2,212	1.7
chrysene	11	9	7	9	2	17	4	1.1	3,262	2.5
benzo[b]fluoranthene	6	6	6	6	0	4	2	1.4	1,671	1.3
benzo[k]fluoranthene	8	7	7	7	1	7	3	1.3	2,213	1.7
benzo[a]pyrene	6	5	4	5	1	11	2	1.1	1,882	1.5
indeno[123cd]pyrene	3	5	2	3	1	27	1	1.1	1,271	1.0
dibenz[ah]anthracene	ND	2	3	2	---	---	1	1.1	770	0.6
benzo[ghi]perylene	3	3	2	3	1	23	1	1.9	566	0.4

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 C. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(Low Water Concentration, 1 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	5	5	2	4	1	33	2	1.1	1,369	0.5
acenaphthene	4	6	ND	5	---	---	2	1.5	1,263	0.5
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	ND	ND	ND	---	---	ND	1.0	ND	ND
anthracene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyrene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	24	22	12	19	5	27	8	1.1	7,050	2.7
chrysene	ND	ND	ND	ND	---	---	ND	1.1	ND	ND
benzo[b]fluoranthene	24	21	ND	23	---	---	9	1.4	6,495	2.5
benzo[k]fluoranthene	29	25	ND	27	---	---	11	1.3	8,390	3.3
benzo[a]pyrene	27	25	18	23	4	15	9	1.1	8,590	3.3
indeno[123cd]pyrene	20	19	10	17	4	27	7	1.1	6,073	2.4
dibenz[ah]anthracene	20	15	6	14	6	42	6	1.1	5,034	2.0
benzo[ghi]perylene	22	12	7	14	7	47	6	1.9	2,915	1.1

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 D. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(Low Water Concentration, 1 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	1.2	ND	ND
acenaphthylene	6	3	6	5	1	29	2	1.1	1,686	0.4
acenaphthene	7	5	7	6	1	18	3	1.5	1,694	0.4
fluorene	ND	ND	ND	ND	---	---	ND	0.5	ND	ND
phenanthrene	ND	NG	ND	ND	---	---	ND	1.0	ND	ND
anthracene	11	ND	ND	11	---	---	4	1.1	3,996	1.0
fluoranthene	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
pyreue	ND	ND	ND	ND	---	---	ND	1.3	ND	ND
benz[a]anthracene	27	14	25	22	6	26	9	1.1	8,003	2.1
chryseue	25	13	27	22	6	30	9	1.1	7,967	2.1
benzo[b]fluoranthene	24	ND	22	23	---	---	9	1.4	6,567	1.7
benzo[k]fluoranthene	34	21	40	31	8	25	13	1.3	9,760	2.5
benzo[a]pyrene	31	20	30	27	5	19	11	1.1	9,861	2.6
indeno[123cd]pyrene	22	13	20	18	4	21	7	1.1	6,635	1.7
dibenz[ah]anthracene	20	9	18	16	5	31	6	1.1	5,792	1.5
benzo[ghi]perylene	18	12	15	15	3	18	6	1.9	3,205	0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 E. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(Medium Water Concentration, 10 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	10	10	9	10	1	7	4	9	442	0.6
acenaphthene	24	24	21	23	1	5	9	11	810	1.1
fluorene	20	17	19	18	1	7	7	8	973	1.3
phenanthrene	45	47	45	46	1	2	18	8	2,383	3.3
anthracene	42	45	39	42	3	6	17	10	1,743	2.4
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	60	62	51	57	5	8	23	8	3,004	4.1
benz[a]anthracene	54	58	62	58	3	5	23	10	2,245	3.1
chrysene	62	76	82	73	8	12	29	11	2,577	3.5
benzo[b]fluoranthene	55	54	48	52	3	6	21	12	1,726	2.4
benzo[k]fluoranthene	68	75	72	72	3	4	29	12	2,354	3.2
benzo[a]pyrene	60	54	58	57	3	5	23	12	1,994	2.7
indeno[123cd]pyrene	43	41	35	40	3	8	16	11	1,521	2.1
dibenz[ah]anthracene	34	44	36	38	5	12	15	11	1,407	1.9
benzo[ghi]perylene	26	37	28	31	5	16	12	15	842	1.1

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 F. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(Medium Water Concentration, 10 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	10	10	9	10	0	3	4	9	446	0.3
acenaphthene	23	26	23	24	1	5	10	11	851	0.7
fluorene	24	27	24	25	1	5	10	8	1,335	1.0
phenanthrene	64	67	62	64	2	4	26	8	3,364	2.6
anthracene	59	60	56	58	2	3	24	10	2,424	1.9
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	67	84	77	76	7	9	31	8	3,973	3.1
benz[a]anthracene	94	88	83	88	5	6	36	10	3,418	2.7
chrysene	131	126	118	125	6	4	50	11	4,419	3.4
benzo[b]fluoranthene	82	83	78	81	2	3	33	12	2,667	2.1
benzo[k]fluoranthene	112	98	95	102	7	7	41	12	3,341	2.6
benzo[a]pyrene	83	80	76	79	3	3	32	12	2,761	2.2
indeno[123cd]pyrene	63	63	56	61	3	5	25	11	2,334	1.8
dibenz[ah]anthracene	63	64	62	63	1	1	25	11	2,326	1.8
benzo[ghi]perylene	63	64	63	63	0	1	26	15	1,747	1.4

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 G. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C (Medium Water Concentration, 10 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	14	14	13	14	1	4	6	9	637	0.2
acenaphthene	33	32	34	33	1	2	13	11	1,164	0.5
fluorene	44	43	40	42	2	4	17	8	2,243	0.9
phenanthrene	115	117	109	114	3	3	46	8	5,945	2.3
anthracene	95	107	95	99	6	6	40	10	4,110	1.6
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	171	186	159	172	11	7	69	8	9,012	3.5
benz[a]anthracene	185	204	174	187	12	7	76	10	7,266	2.8
chrysene	208	215	206	210	4	2	85	11	7,423	2.9
benzo[b]fluoranthene	163	179	144	162	14	9	65	12	5,360	2.1
benzo[k]fluoranthene	206	183	179	189	12	6	76	12	6,207	2.4
benzo[a]pyrene	161	168	146	158	9	6	64	12	5,500	2.1
indeno[123cd]pyrene	116	122	111	116	4	4	47	11	4,467	1.7
dibenz[ah]anthracene	114	122	107	114	6	5	46	11	4,222	1.6
benzo[ghi]perylene	130	135	122	129	5	4	52	15	3,562	1.4

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 H. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(Medium Water Concentration, 10 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	ND [4]	ND	ND	ND	---	---	ND	12	ND	ND
acenaphthylene	15	11	18	15	3	21	6	9	677	0.2
acenaphthene	38	29	34	34	4	12	14	11	1,190	0.3
fluorene	40	31	43	38	5	14	15	8	2,014	0.5
phenanthrene	111	87	125	108	16	15	43	8	5,642	1.5
anthracene	100	85	100	95	7	7	38	10	3,945	1.0
fluoranthene	ND	ND	ND	ND	---	---	ND	11	ND	ND
pyrene	193	160	182	178	14	8	72	8	9,332	2.4
benz[a]anthracene	194	165	207	188	18	9	76	10	7,299	1.9
chrysene	222	188	240	217	22	10	87	11	7,660	2.0
benzo[b]fluoranthene	171	143	177	164	15	9	66	12	5,408	1.4
benzo[k]fluoranthene	207	178	217	200	16	8	81	12	6,570	1.7
benzo[a]pyrene	179	154	191	175	16	9	70	12	6,066	1.6
indeno[123cd]pyrene	123	109	133	122	10	8	49	11	4,670	1.2
dibenz[ah]anthracene	117	105	129	117	9	8	47	11	4,326	1.1
benzo[ghi]perylene	135	115	144	131	12	9	53	15	3,627	0.9

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).
[4] ND = (< MQL of Control SPMD).

TABLE 6 I. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(High Water Concentration, 100 ng/L nominal, Day 4)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	22	22	51	32	14	44	13	79	161	0.2
acenaphthylene	176	168	185	176	7	4	71	103	691	0.9
acenaphthene	272	233	267	257	18	7	104	103	1,006	1.4
fluorene	239	230	235	235	3	1	95	89	1,061	1.4
phenanthrene	538	496	535	523	19	4	211	90	2,350	3.2
anthracene	490	853	526	623	164	26	251	113	2,229	3.0
fluoranthene	669	639	716	674	32	5	272	94	2,881	3.9
pyrene	678	692	698	690	8	1	278	85	3,275	4.5
benz[a]anthracene	509	468	469	482	19	4	194	104	1,874	2.6
chrysene	652	661	849	720	91	13	291	103	2,810	3.8
benzo[b]fluoranthene	499	500	556	518	27	5	209	123	1,700	2.3
benzo[k]fluoranthene	499	500	556	518	27	5	209	118	1,766	2.4
benzo[a]pyrene	559	537	589	561	21	4	226	99	2,287	3.1
indeno[123cd]pyrene	383	354	373	370	12	3	149	93	1,597	2.2
dibenz[ah]anthracene	370	362	425	386	28	7	155	108	1,434	2.0
benzo[ghi]perylene	353	374	337	355	15	4	143	126	1,131	1.5

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).

TABLE 6 J. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(High Water Concentration, 100 ng/L nominal, Day 7)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	38	---	26	32	---	---	13	79	163	0.1
acenaphthylene	259	178	220	219	33	15	88	103	858	0.7
acenaphthene	401	266	315	327	56	17	132	103	1,280	1.0
fluorene	389	263	271	308	58	19	124	89	1,391	1.1
phenanthrene	803	713	703	739	45	6	298	90	3,324	2.6
anthracene	795	698	694	729	47	6	294	113	2,609	2.0
fluoranthene	951	852	904	902	40	4	364	94	3,854	3.0
pyrene	995	908	936	946	37	4	382	85	4,495	3.5
benz[a]anthracene	782	660	731	724	50	7	292	104	2,816	2.2
chrysene	1,336	1,291	1,192	1,273	60	5	513	103	4,964	3.9
benzo[b]fluoranthene	426	336	521	428	75	18	172	123	1,403	1.1
benzo[k]fluoranthene	1,375	1,322	1,248	1,315	52	4	530	118	4,482	3.5
benzo[a]pyrene	914	813	793	840	53	6	339	99	3,421	2.7
indeno[123cd]pyrene	604	---	589	597	---	---	241	93	2,577	2.0
dibenz[ah]anthracene	535	875	593	667	148	22	269	108	2,482	1.9
benzo[ghi]perylene	617	647	564	609	34	6	246	126	1,943	1.5

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).

TABLE 6 K. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C
(High Water Concentration, 100 ng/L nominal, Day 14)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	24	24	26	25	1	4	10	79	127	0.0
acenaphthylene	291	299	290	293	4	1	118	103	1,150	0.4
acenaphthene	478	496	462	478	14	3	193	103	1,871	0.7
fluorene	565	691	679	645	56	9	260	89	2,915	1.1
phenanthrene	1,371	1,552	1,528	1,484	80	5	598	90	6,670	2.6
anthracene	1,282	1,363	1,454	1,366	70	5	551	113	4,888	1.9
fluoranthene	2,002	2,237	2,455	2,231	185	8	900	94	9,531	3.7
pyrene	2,004	2,342	2,509	2,285	210	9	921	85	10,853	4.2
benz[a]anthracene	1,823	2,194	2,170	2,062	170	8	832	104	8,019	3.1
chrysene	2,107	2,499	2,638	2,414	225	9	974	103	9,415	3.7
benzo[b]fluoranthene	1,382	1,165	1,854	1,467	288	20	591	123	4,812	1.9
benzo[k]fluoranthene	2,269	3,077	2,609	2,652	331	12	1,069	118	9,039	3.5
benzo[a]pyrene	1,718	1,987	2,117	1,940	166	9	782	99	7,903	3.1
indeno[123cd]pyrene	1,141	1,331	1,394	1,289	108	8	520	93	5,563	2.2
dibenz[ah]anthracene	1,052	1,229	1,270	1,183	95	8	477	108	4,402	1.7
benzo[ghi]perylene	1,225	1,470	1,416	1,370	105	8	552	126	4,371	1.7

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).

TABLE 6 L. SPMD UPTAKE OF PAHS IN FLOW-THROUGH DILUTER EXPOSURES AT 26 °C (High Water Concentration, 100 ng/L nominal, Day 21)

PAHs	ng / SPMD [1]						Mean SPMD [1] Conc. ng/g	Mean Water Conc. ng/L	Concentration Factor [2] SPMD / Water	SPMD-Rs [3] Liters per Day
	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.				
naphthalene	33	23	28	28	4	14	11	79	143	0.0
acenaphthylene	351	299	228	293	50	17	118	103	1,148	0.3
acenaphthene	612	492	365	489	101	21	197	103	1,914	0.5
fluorene	779	709	489	659	124	19	266	89	2,979	0.8
phenanthrene	1,936	1,794	1,272	1,667	286	17	672	90	7,495	1.9
anthracene	1,603	1,559	1,161	1,441	199	14	581	113	5,156	1.3
fluoranthene	3,160	3,001	2,206	2,789	417	15	1,125	94	11,913	3.1
pyrene	3,189	2,985	2,233	2,802	411	15	1,130	85	13,309	3.5
benz[a]anthracene	2,796	2,802	1,848	2,482	448	18	1,001	104	9,650	2.5
chrysene	3,131	3,055	2,429	2,872	315	11	1,158	103	11,198	2.9
benzo[b]fluoranthene	2,693	2,275	1,541	2,170	477	22	875	123	7,118	1.8
benzo[k]fluoranthene	3,121	3,329	2,573	3,008	319	11	1,213	118	10,253	2.7
benzo[a]pyrene	2,808	2,786	1,950	2,515	399	16	1,014	99	10,242	2.7
indeno[123cd]pyrene	2,001	1,817	1,191	1,670	347	21	673	93	7,209	1.9
dibenz[ah]anthracene	1,659	1,581	1,099	1,446	248	17	583	108	5,380	1.4
benzo[ghi]perylene	1,796	1,697	1,278	1,590	225	14	641	126	5,073	1.3

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Concentration Factor derived by dividing (ng analyte/g SPMD by ng analyte/g water
[3] SPMD sampling rate normalized to a 1g triolein configuration (membrane = 4.45g).

TABLE 7 A. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Membranes, Day 1).

PAHs	ng / Membrane [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	41	35	44	40	92	78	97	89	8	9
acenaphthylene	45	62	69	59	101	137	154	130	22	17
acenaphthene	54	65	91	70	119	145	203	156	35	22
fluorene	53	77	88	73	117	171	196	161	33	20
phenanthrene	69	128	131	109	153	284	291	243	63	26
anthracene	37	75	74	62	81	167	165	138	40	29
fluoranthene	148	142	149	146	330	315	330	325	7	2
pyrene	258	133	128	173	572	296	285	384	133	35
benz[a]anthracene	76	132	129	113	169	294	286	250	57	23
chrysene	61	162	111	111	135	360	247	247	92	37
benzo[b]fluoranthene	75	131	134	113	166	290	299	251	61	24
benzo[k]fluoranthene	68	154	136	119	151	341	301	264	82	31
benzo[a]pyrene	81	96	106	94	180	214	235	210	23	11
indeno[123cd]pyrene	92	110	84	95	204	244	187	212	24	11
dibenz[ah]anthracene	76	38	28	47	168	84	61	104	46	44
benzo[ghi]perylene	83	37	27	49	184	83	60	109	54	49

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 7 B. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Membranes, Day 4).

PAHs	ng / Membrane [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	50	43	52	48	111	96	114	107	8.1	8
acenaphthylene	125	136	137	133	277	303	304	295	12.4	4
acenaphthene	166	183	187	179	369	406	416	397	20.4	5
fluorene	167	193	169	176	371	428	374	391	26.3	7
phenanthrene	257	275	257	263	570	610	570	584	19.0	3
anthracene	234	245	214	231	519	544	476	513	28.2	5
fluoranthene	352	323	345	340	782	717	766	755	27.6	4
pyrene	303	300	296	299	672	666	657	665	6.4	1
benz[a]anthracene	280	273	280	277	622	606	622	617	7.6	1
chrysene	341	303	303	316	758	674	673	701	39.9	6
benzo[b]fluoranthene	277	280	296	284	614	623	658	632	18.8	3
benzo[k]fluoranthene	346	319	358	341	768	708	796	757	36.8	5
benzo[a]pyrene	244	245	231	240	543	544	514	534	13.8	3
indeno[123cd]pyrene	211	208	236	218	469	462	524	485	27.6	6
dibenz[ah]anthracene	58	57	65	60	128	126	145	133	8.2	6
benzo[ghi]perylene	75	55	80	70	166	123	177	155	23.3	15

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 7 C. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Membranes, Day 7).

PAHs	ng / Membrane [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	29	31	29	29	65	68	64	65	1.8	3
acenaphthylene	96	98	92	95	212	217	205	211	5.0	2
acenaphthene	150	143	139	144	333	317	308	319	10.3	3
fluorene	202	146	192	180	450	324	426	400	54.3	14
phenanthrene	241	220	215	225	535	489	478	501	24.7	5
anthracene	218	192	190	200	485	426	422	444	29.0	7
fluoranthene	350	296	284	310	777	658	630	689	63.6	9
pyrene	416	259	244	306	925	576	541	681	173.3	25
benz[a]anthracene	315	287	262	288	701	638	583	641	48.2	8
chrysene	336	289	280	302	747	643	622	671	54.5	8
benzo[b]fluoranthene	346	333	291	323	768	740	646	718	52.3	7
benzo[k]fluoranthene	344	302	293	313	765	670	651	695	50.0	7
benzo[a]pyrene	294	273	255	274	653	606	566	608	35.4	6
indeno[123cd]pyrene	287	275	248	270	638	610	552	600	35.7	6
dibenz[ah]anthracene	52	63	54	56	116	139	120	125	10.3	8
benzo[ghi]perylene	146	114	85	115	325	252	189	255	55.4	22

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 7 D. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Membranes, Day 14).

PAHs	ng / Membrane [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	35	32	40	35	77	71	89	79	7.5	9
acenaphthylene	115	111	117	114	256	246	260	254	5.8	2
acenaphthene	179	172	184	178	398	381	408	396	11.2	3
fluorene	199	195	207	200	443	433	460	445	11.4	3
phenanthrene	262	264	266	264	582	587	591	587	3.8	1
anthracene	251	242	238	244	559	538	529	542	12.5	2
fluoranthene	352	365	372	363	783	812	826	807	17.9	2
pyrene	343	367	557	422	762	814	1,237	938	212.6	23
benz[a]anthracene	432	371	355	386	960	824	788	857	73.8	9
chrysene	408	356	343	369	906	792	763	820	61.9	8
benzo[b]fluoranthene	415	381	378	391	923	846	840	869	37.9	4
benzo[k]fluoranthene	442	366	355	387	982	812	789	861	86.0	10
benzo[a]pyrene	316	317	313	315	701	704	695	700	3.7	1
indeno[123cd]pyrene	361	353	368	360	802	784	817	801	13.5	2
dibenz[ah]anthracene	111	109	110	110	247	243	244	245	1.8	1
benzo[ghi]perylene	172	178	192	180	381	394	426	401	18.8	5

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 7 E. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Membranes, Day 28).

PAHs	ng / Membrane [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	27	23	37	29	61	52	83	65	12.9	20
acenaphthylene	90	68	88	82	199	151	195	182	21.8	12
acenaphthene	159	105	141	135	354	234	313	300	49.9	17
fluorene	179	120	167	155	398	266	370	345	56.9	16
phenanthrene	225	163	220	202	499	362	488	450	62.2	14
anthracene	192	103	175	156	426	229	388	348	85.3	25
fluoranthene	350	226	335	304	778	503	745	675	122.5	18
pyrene	412	211	371	331	916	468	824	736	192.9	26
benz[a]anthracene	386	206	319	303	858	457	708	674	165.4	25
chrysene	337	229	319	295	750	509	708	655	105.2	16
benzo[b]fluoranthene	370	239	362	324	821	532	805	719	132.6	18
benzo[k]fluoranthene	383	246	337	322	851	546	748	715	126.6	18
benzo[a]pyrene	302	179	339	273	670	397	752	607	151.9	25
indeno[123cd]pyrene	343	260	388	330	763	578	863	734	118.0	16
dibenz[ah]anthracene	206	73	154	144	457	163	341	320	121.0	38
benzo[ghi]perylene	290	225	256	257	645	501	568	571	59.0	10

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 7 F. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Membranes, Day 56).

PAHs	ng / Membrane [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	27	13	26	22	59	29	57	48	13.8	29
acenaphthylene	81	61	103	82	180	136	228	182	37.6	21
acenaphthene	135	141	164	147	299	314	364	326	28.0	9
fluorene	179	157	211	182	397	350	469	405	49.0	12
phenanthrene	219	189	270	226	486	420	599	502	73.7	15
anthracene	180	142	162	161	401	315	361	359	35.0	10
fluoranthene	296	307	416	340	658	682	925	755	120.6	16
pyrene	309	298	461	356	686	662	1,023	790	165.0	21
benz[a]anthracene	431	341	417	396	959	758	926	881	87.8	10
chrysene	443	308	351	367	984	684	780	816	125.3	15
benzo[b]fluoranthene	404	357	428	397	899	794	951	881	65.5	7
benzo[k]fluoranthene	446	348	399	397	991	773	886	883	89.2	10
benzo[a]pyrene	281	279	337	299	624	620	749	664	60.0	9
indeno[123cd]pyrene	300	276	349	308	666	614	776	685	67.3	10
dibenz[ah]anthracene	229	243	310	260	508	540	689	579	78.7	14
benzo[ghi]perylene	374	291	433	366	832	646	962	813	129.8	16

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 8 A. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Lipid, Day 1).

PAHs	ng / Lipid [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	77	90	118	95	846	991	1,293	1,044	186.3	18
acenaphthylene	88	99	143	110	965	1,090	1,573	1,209	262.0	22
acenaphthene	97	98	158	118	1,065	1,078	1,741	1,295	315.5	24
fluorene	96	95	182	124	1,057	1,047	1,997	1,367	445.3	33
phenanthrene	126	131	196	151	1,388	1,435	2,157	1,660	352.0	21
anthracene	78	87	125	97	854	955	1,374	1,061	225.0	21
fluoranthene	99	96	151	116	1,088	1,059	1,664	1,270	278.4	22
pyrene	84	90	127	100	920	986	1,392	1,099	209.0	19
benz[a]anthracene	85	91	128	101	931	1,001	1,409	1,114	210.7	19
chrysene	62	77	131	90	682	844	1,438	988	325.1	33
benzo[b]fluoranthene	59	61	89	70	652	670	975	766	148.3	19
benzo[k]fluoranthene	56	42	89	62	620	464	975	686	213.8	31
benzo[a]pyrene	47	41	68	52	518	451	745	571	126.0	22
indeno[123cd]pyrene	13	10	<10	11	145	114	<110	123	---	---
dibenz[ah]anthracene	<10 [2]	<10	<10	<10	<110	<110	<110	<110	---	---
benzo[ghi]perylene	<12	<12	<12	<12	<128	<128	<128	<128	---	---

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).
[2] < = <MQL of Control Lipid on Day 1.

TABLE 8 B. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Lipid, Day 4).

PAHs	ng / Lipid [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	88	74	93	85	964	808	1,025	932	91.6	10
acenaphthylene	140	133	152	142	1,533	1,466	1,675	1,558	87.0	6
acenaphthene	160	150	173	161	1,758	1,651	1,904	1,771	104.0	6
fluorene	195	202	213	203	2,140	2,224	2,344	2,236	83.9	4
phenanthrene	194	191	212	199	2,131	2,099	2,331	2,187	102.6	5
anthracene	147	148	184	160	1,612	1,623	2,023	1,753	191.2	11
fluoranthene	168	164	198	177	1,849	1,798	2,173	1,940	165.8	9
pyrene	148	151	167	155	1,627	1,654	1,840	1,707	94.4	6
benz[a]anthracene	169	167	189	175	1,856	1,837	2,073	1,922	106.7	6
chrysene	136	154	152	147	1,498	1,693	1,668	1,620	86.9	5
benzo[b]fluoranthene	134	127	152	137	1,473	1,391	1,668	1,511	116.2	8
benzo[k]fluoranthene	74	88	90	84	814	970	991	925	78.9	9
benzo[a]pyrene	82	80	97	86	898	875	1,065	946	84.7	9
indeno[123cd]pyrene	49	47	63	53	535	515	688	579	77.1	13
dibenz[ah]anthracene	13	13	15	14	141	141	164	148	10.9	7
benzo[ghi]perylene	39	37	33	36	429	408	357	398	30.0	8

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 8 C. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Lipid, Day 7).

PAHs	ng / Lipid [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	67	99	65	77	736	1,090	710	845	173.4	21
acenaphthylene	123	207	108	146	1,352	2,276	1,181	1,603	480.9	30
acenaphthene	136	248	119	168	1,493	2,726	1,304	1,841	630.5	34
fluorene	171	273	143	196	1,880	3,002	1,568	2,150	615.8	29
phenanthrene	183	284	161	209	2,011	3,122	1,770	2,301	588.7	26
anthracene	137	256	111	168	1,508	2,808	1,216	1,844	691.8	38
fluoranthene	183	272	151	202	2,015	2,991	1,657	2,221	563.7	25
pyrene	146	226	127	166	1,609	2,482	1,397	1,829	469.9	26
benz[a]anthracene	157	226	139	174	1,720	2,479	1,522	1,907	412.6	22
chrysene	156	226	144	175	1,713	2,481	1,578	1,924	397.8	21
benzo[b]fluoranthene	133	195	122	150	1,462	2,147	1,343	1,651	354.5	21
benzo[k]fluoranthene	102	160	87	116	1,123	1,756	958	1,279	343.9	27
benzo[a]pyrene	79	129	76	95	873	1,416	835	1,041	265.7	26
indeno[123cd]pyrene	54	87	51	64	596	953	556	701	178.4	25
dibenz[ah]anthracene	17	35	16	23	186	386	175	249	97.0	39
benzo[ghi]perylene	19	34	13	22	203	375	144	241	97.8	41

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 8 D. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Lipid, Day 14).

PAHs	ng / Lipid [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	84	14 [2]	55	70	927	151	601	764	---	---
acenaphthylene	169	87 [2]	149	159	1,853	951	1,635	1,744	---	---
acenaphthene	197	108 [2]	175	186	2,166	1,184	1,921	2,043	---	---
fluorene	232	175	208	205	2,548	1,926	2,282	2,252	254.8	11
phenanthrene	265	211	236	237	2,915	2,314	2,595	2,608	245.6	9
anthracene	213	151	172	179	2,344	1,658	1,885	1,962	285.3	15
fluoranthene	269	219	237	241	2,952	2,405	2,602	2,653	225.9	9
pyrene	220	188	199	202	2,419	2,062	2,184	2,221	148.2	7
benz[a]anthracene	270	205	221	232	2,967	2,252	2,431	2,550	303.9	12
chrysene	235	181	205	207	2,578	1,991	2,256	2,275	239.9	11
benzo[b]fluoranthene	196	168	180	181	2,149	1,845	1,977	1,990	124.6	6
benzo[k]fluoranthene	182	144	151	159	1,997	1,585	1,662	1,748	178.9	10
benzo[a]pyrene	134	108	121	121	1,470	1,187	1,333	1,330	115.8	9
indeno[123cd]pyrene	99	82	85	89	1,090	901	931	974	83.0	9
dibenz[ah]anthracene	45	36	36	39	489	390	398	426	44.9	11
benzo[ghi]perylene	40	34	32	35	442	376	347	388	39.6	10

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).
[2] = Compounds lost during sample processing, not used in calculaltions.

TABLE 8 E. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Lipid, Day 28).

PAHs	ng / Lipid [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	32	99	84	72	349	1,084	925	786	315.4	40
acenaphthylene	112	199	169	160	1,229	2,191	1,853	1,758	398.7	23
acenaphthene	165	258	216	213	1,810	2,830	2,376	2,338	417.2	18
fluorene	202	290	234	242	2,214	3,190	2,567	2,657	403.4	15
phenanthrene	240	339	255	278	2,632	3,723	2,804	3,053	478.9	16
anthracene	220	233	221	225	2,421	2,556	2,430	2,469	61.7	3
fluoranthene	295	384	252	310	3,236	4,218	2,768	3,407	604.0	18
pyrene	250	341	214	268	2,742	3,748	2,352	2,947	588.4	20
benz[a]anthracene	283	313	236	277	3,104	3,437	2,590	3,044	348.5	11
chrysene	271	290	246	269	2,980	3,189	2,704	2,958	198.5	7
benzo[b]fluoranthene	221	254	187	221	2,427	2,790	2,057	2,425	299.2	12
benzo[k]fluoranthene	204	215	165	195	2,241	2,363	1,815	2,140	234.6	11
benzo[a]pyrene	161	177	126	154	1,765	1,944	1,384	1,697	233.7	14
indeno[123cd]pyrene	126	138	93	119	1,387	1,520	1,021	1,309	210.9	16
dibenz[ah]anthracene	120	55	53	76	1,320	602	579	834	343.8	41
benzo[ghi]perylene	165	115	61	114	1,813	1,267	671	1,251	466.3	37

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).

TABLE 8 F. SPMD UPTAKE OF PAHs IN FRESHWATER STATIC EXPOSURES (SPMD Lipid, Day 56).

PAHs	ng / Lipid [1]				ng/g					
	Rep #1	Rep #2	Rep #3	Mean	Rep #1	Rep #2	Rep #3	Mean	Std Dev.	C.V.
naphthalene	41	22	<10 [2,3]	32	453	244	<110	348	---	---
acenaphthylene	121	92	58 [3]	107	1,330	1,014	640	1,172	---	---
acenaphthene	164	142	91 [3]	153	1,803	1,560	997	1,682	---	---
fluorene	215	190	161	189	2,358	2,087	1,774	2,073	238.9	12
phenanthrene	263	209	218	230	2,890	2,301	2,391	2,527	259.0	10
anthracene	196	171	135	167	2,151	1,874	1,484	1,836	273.6	15
fluoranthene	275	259	225	253	3,019	2,848	2,471	2,779	228.7	8
pyrene	231	208	192	210	2,533	2,290	2,110	2,311	173.4	8
benz[a]anthracene	317	223	262	267	3,478	2,449	2,879	2,936	421.8	14
chrysene	259	242	233	245	2,843	2,656	2,563	2,687	116.5	4
benzo[b]fluoranthene	225	189	171	195	2,473	2,077	1,877	2,142	247.5	12
benzo[k]fluoranthene	228	177	182	196	2,508	1,943	1,996	2,149	254.7	12
benzo[a]pyrene	155	125	113	131	1,701	1,377	1,238	1,439	193.9	13
indeno[123cd]pyrene	116	86	82	95	1,276	940	902	1,039	168.0	16
dibenz[ah]anthracene	103	82	75	87	1,133	897	823	951	132.2	14
benzo[ghi]perylene	130	91	89	103	1,429	998	974	1,133	209.0	18

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).
[2] < = <MQL of Control Lipid on Day 56.
[3] = Compounds lost during sample processing, not used in calculaltions.

TABLE 9 A. WATER CONCENTRATIONS FOR PAH FRESHWATER STATIC EXPOSURES, DAY 1

PAHs	ng / Sample [1]						ng / L [2]
	Rep 1	Rep 2	Rep 3	Mean	Std Dev.	C.V.	Mean
naphthalene	175	507	433	372	142	38	530
acenaphthylene	456	515	483	485	24	5	598
aceuaphthene	406	511	464	461	43	9	595
fluorene	415	512	440	456	41	9	550
phenanthrene	460	466	450	458	7	1	536
anthracene	460	454	405	439	24	6	660
fluoranthene	474	493	470	479	10	2	502
pyrene	491	473	448	470	17	4	402
benz[a]anthracene	497	488	462	482	15	3	536
chrysene	497	540	514	517	18	3	618
benzo[b]fluoranthene	463	464	434	453	14	3	450
benzo[k]fluoranthene	463	442	430	445	14	3	618
benzo[a]pyrene	397	378	355	377	17	5	455
indeno[123cd]pyrene	377	396	386	386	8	2	452
dibenz[ah]anthracene	282	284	293	286	5	2	448
benzo[ghi]perylene	243	286	282	270	19	7	493

[1] Sample = 0.9 L of water extracted.

[2] ng/L results corrected for water spike recovery.

TABLE 9 B. WATER CONCENTRATIONS FOR PAH FRESHWATER STATIC EXPOSURES, DAY 4

PAHs	ng / Sample [1]						ng / L [2]
	Rep 1	Rep 2	Rep 3	Mean	Std Dev.	C.V.	Mean
naphthalene	350	113	398	287	124	43	409
acenaphthylene	255	204	300	253	39	16	312
acenaphthene	208	178	252	213	31	14	275
fluorene	188	210	209	202	10	5	244
phenanthrene	153	182	168	168	12	7	196
anthracene	153	134	149	145	8	6	218
fluoranthene	137	191	182	170	24	14	178
pyrene	152	156	134	147	10	7	126
benz[a]anthracene	190	188	156	178	16	9	198
chrysene	190	205	213	203	9	5	242
benzo[b]fluoranthene	183	181	148	171	16	9	169
benzo[k]fluoranthene	183	235	227	215	23	11	298
benzo[a]pyrene	142	155	155	151	6	4	182
indeno[123cd]pyrene	247	249	232	243	8	3	284
dibenz[ah]anthracene	266	288	295	283	13	4	443
benzo[ghi]perylene	261	247	246	251	7	3	458

[1] Sample = 0.9 L of water extracted.
[2] ng/L results corrected for water spike recovery.

TABLE 9 C. WATER CONCENTRATIONS FOR PAH FRESHWATER STATIC EXPOSURES, DAY 7

PAHs	ng / Sample [1]						ng / L [2]
	Rep 1	Rep 2	Rep 3	Mean	Std Dev.	C.V.	Mean
naphthalene	305	229	206	246	42	17	351
acenaphthylene	249	195	170	204	33	16	252
acenaphthene	191	142	123	152	29	19	197
fluorene	204	125	110	147	41	28	177
phenanthrene	83	77	70	77	5	7	90
anthracene	83	49	44	59	17	30	88
fluoranthene	52	46	48	48	2	5	51
pyrene	34	42	53	43	8	18	37
benz[a]anthracene	20	44	48	38	13	33	42
chrysene	20	44	50	38	13	34	45
benzo[b]fluoranthene	92	47	50	63	20	32	62
benzo[k]fluoranthene	92	64	69	75	12	16	104
benzo[a]pyrene	76	36	42	51	18	35	62
indeno[123cd]pyrene	126	82	83	97	21	21	113
dibenz[ah]anthracene	213	165	150	176	27	15	276
benzo[ghi]perylene	118	144	131	131	11	8	238

[1] Sample = 0.9 L of water extracted.
[2] ng/L results corrected for water spike recovery.

TABLE 9 D. WATER CONCENTRATIONS FOR PAH FRESHWATER STATIC EXPOSURES, DAY 14

PAHs	ng / Sample [1]						ng / L [2]
	Rep 1	Rep 2	Rep 3	Mean	Std Dev.	C.V.	Mean
naphthalene	222	---[3]	32	127	---	---	181
acenaphthylene	201	---	66	133	---	---	164
acenaphthene	117	---	32	75	---	---	96
fluorene	112	---	71	91	---	---	110
phenanthrene	60	---	53	57	---	---	66
anthracene	42	---	35	38	---	---	58
fluoranthene	21	---	23	22	---	---	23
pyrene	21	---	15	18	---	---	15
benz[a]anthracene	25	---	23	24	---	---	27
chrysene	28	---	23	26	---	---	31
benzo[b]fluoranthene	24	---	16	20	---	---	20
benzo[k]fluoranthene	46	---	67	57	---	---	79
benzo[a]pyrene	20	---	28	24	---	---	29
indeno[123cd]pyrene	58	---	64	61	---	---	71
dibenz[ah]anthracene	137	---	212	175	---	---	274
benzo[ghi]perylene	129	---	185	157	---	---	286

[1] Sample = 0.9 L of water extracted.
[2] ng/L results corrected for water spike recovery.
[3] --- = Less than 3 Reps available.

TABLE 9 E. WATER CONCENTRATIONS FOR PAH FRESHWATER STATIC EXPOSURES, DAY 28

PAHs	ng / Sample [1]						ng / L [2]
	Rep 1	Rep 2	Rep 3	Mean	Std Dev.	C.V.	Mean
naphthalene	76	144	172	131	40	31	186
acenaphthylene	79	97	152	109	31	28	135
acenaphthene	74	62	100	79	16	20	102
fluorene	60	55	83	66	12	19	80
phenanthrene	35	30	37	34	3	8	40
anthracene	19	12	37	23	10	45	34
fluoranthene	17	12	64	31	23	75	32
pyrene	13	11	334	12	12	100	10
benz[a]anthracene	11	6	14	11	3	31	12
chrysene	18	4	14	12	6	48	15
benzo[b]fluoranthene	8	<2[3]	9	6	6	100	6
benzo[k]fluoranthene	12	5	9	8	3	31	12
benzo[a]pyrene	7	3	2	4	2	49	5
indeno[123cd]pyrene	32	10	34	25	11	42	29
dibenz[ah]anthracene	115	62	90	89	22	24	140
benzo[ghi]perylene	74	41	63	59	13	23	108

[1] Sample = 0.9 L of water extracted.
[2] ng/L results corrected for water spike recovery.
[3] < = Concentration less than MQL of Control Water.

TABLE 9 F. WATER CONCENTRATIONS FOR PAH FRESHWATER STATIC EXPOSURES, DAY 56

PAHs	ng / Sample [1]						ng / L [2]
	Rep 1	Rep 2	Rep 3	Mean	Std Dev.	C.V.	Mean
naphthalene	133	55	148	112	41	37	159
acenaphthylene	117	75	76	89	19	21	110
acenaphthene	80	59	81	74	10	14	95
fluorene	61	59	58	59	2	3	72
phenanthrene	31	27	---[3]	29	---	---	34
anthracene	14	12	---	13	---	---	19
fluoranthene	22	43	23	29	10	33	31
pyrene	23	62	66	51	19	38	43
benz[a]anthracene	6	5	---	5	---	---	6
chryseue	2	6	---	4	---	---	5
benzo[b]fluoranthene	<2[4]	4	---	3	---	---	3
benzo[k]fluoranthene	<2	4	---	3	---	---	4
benzo[a]pyrene	3	5	---	4	---	---	5
indeno[123cd]pyrene	6	15	---	10	---	---	12
dibenz[ah]anthracene	53	114	75	81	25	31	126
benzo[ghi]perylene	43	109	---	76	---	---	139

[1] Sample = 0.9 L of water extracted.
[2] ng/L results corrected for water spike recovery.
[3] --- = Less than 3 Reps available.
[4] < = Concentration less than MQL of Control Water.

FIGURES

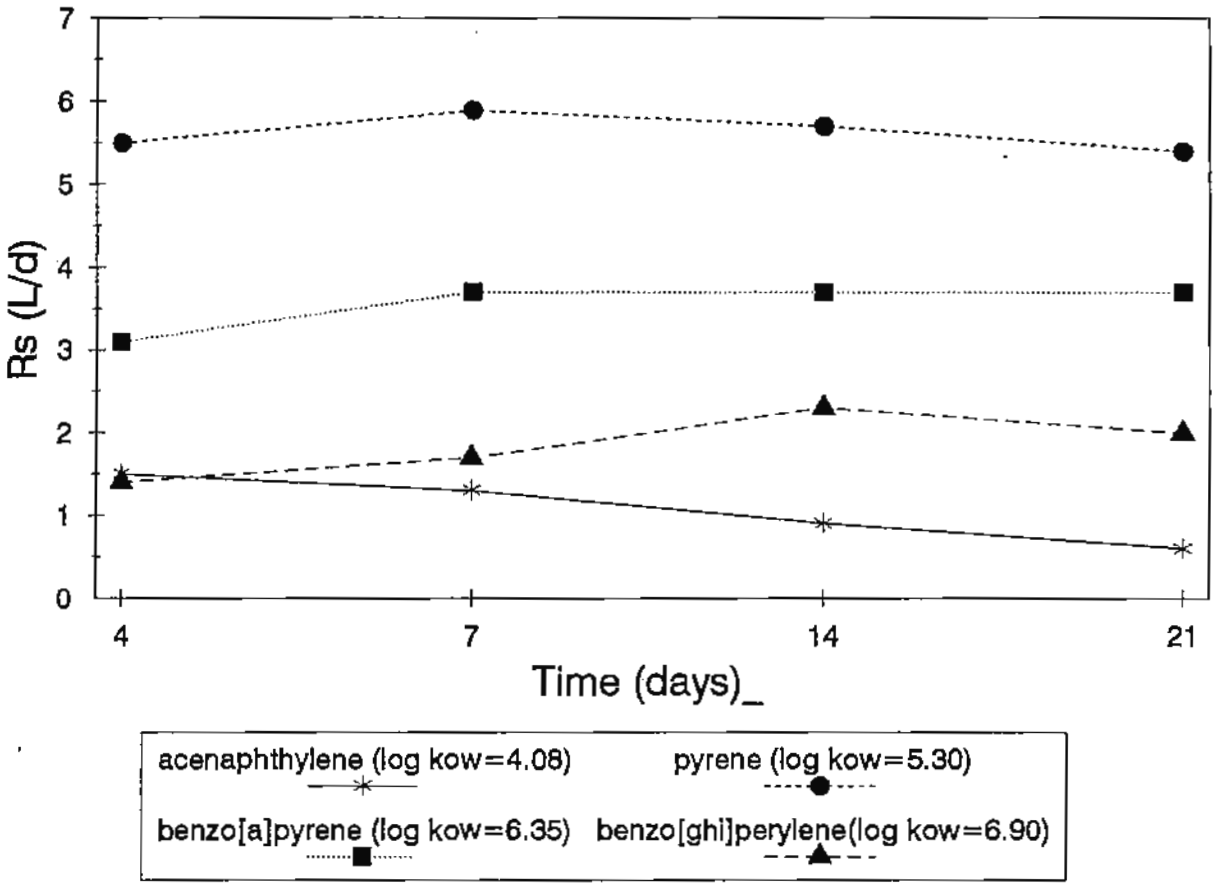

Figure 1. Effect of exposure time on SPMD sampling rates (Rs 1 g triolein configuration) for selected PAHs. Values (flow through, 100 ng/L at 18°C) are recovery corrected and represent the mean of three determinations. In all cases the coefficients of variation are ≤ 10%.

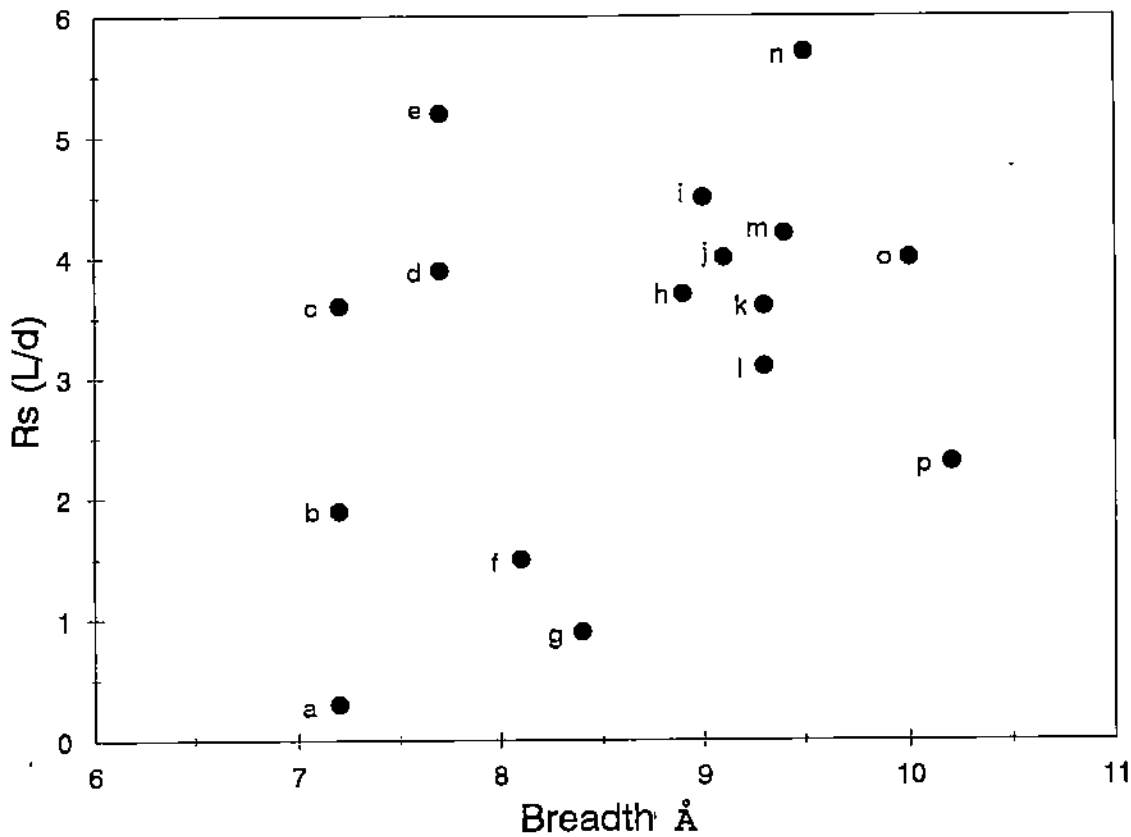

Figure 2. The potential relationship between SPMD sampling rates (Rs, 1 g triolein configuration) and PAH molecular breadth. Data used are recovery corrected and from the 100 ng/L flow-through exposure (18°C) at 14 days. Each Rs value represents mean of three determinations (coefficient of variations <14% for all compounds except naphthalene [46%]) and analytes are designated by letters as follows: a, naphthalene; b, fluorene; c, anthracene; d, phenanthrene; e, chrysene; f, acenaphthene; g, acenaphthylene; h, benzo[a]pyrene; i, fluoranthene; j, benzo[k]fluoranthene; k, benzo[b]fluoranthene; l, dibenz[a,h]anthracene; m, benz[a]anthracene; n, pyrene; o, indeno[1,2,3-cd]pyrene; and p, benzo[g,h,i]perylene.

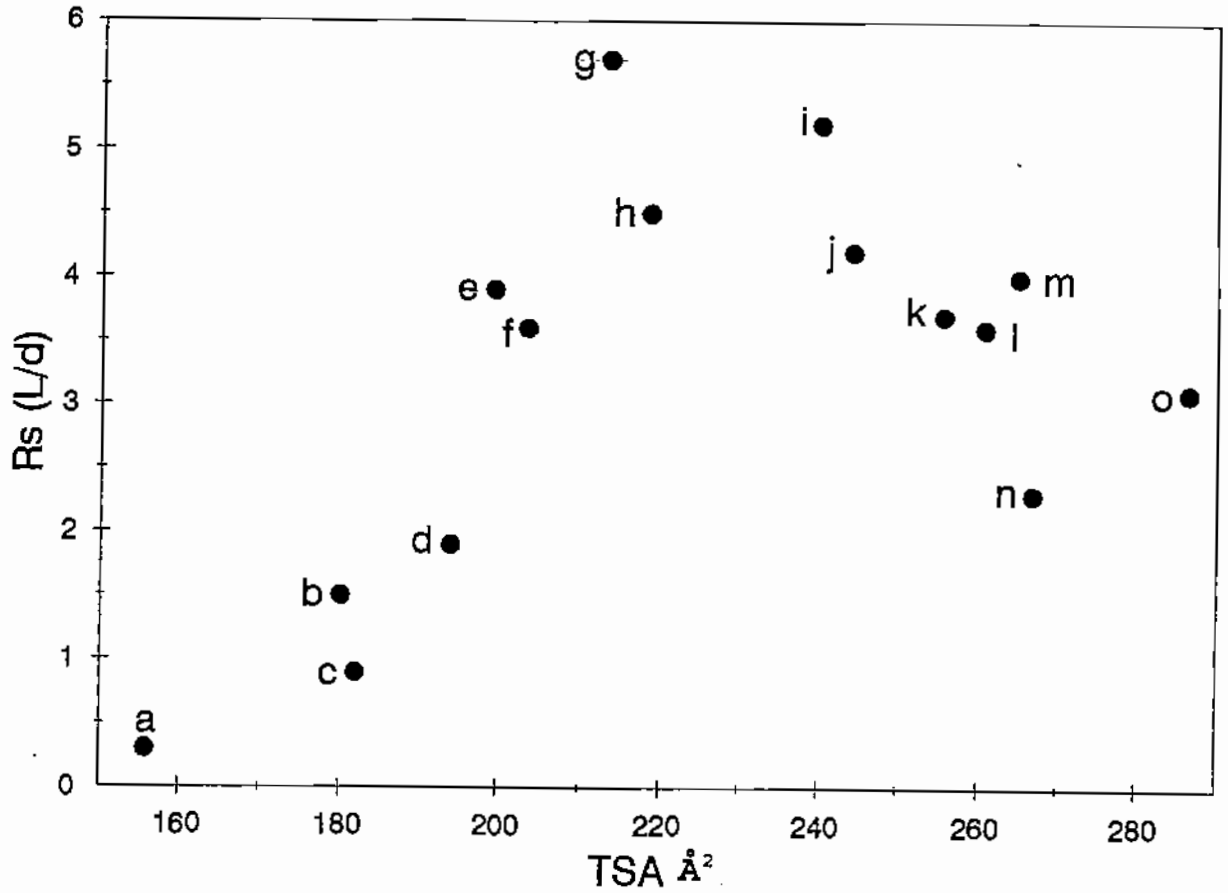

Figure 3 The potential relationship between SPMD sampling rates (Rs, 1 g triolein configuration) and PAH molecular surface area (TSA) in Å². Data used are recovery corrected and from the 100 ng/L flow-through exposure (18°C) at 14 days. Each Rs value represents mean of three determinations (coefficients of variation <14% for all compounds except naphthalene [46%]). Analytes are designated by letters as follows: a, Naphthalene; b, Acenaphthene; c, Acenaphthene; d, Fluorene; e, Phenanthrene; f, Anthracene; g, Pyrene; h, Fluoranthene; i, Chrysene; j, Benz[a]anthracene; k, Benzo[a]pyrene, l, Benzo[b]fluoranthene; m, Benzo[k]fluoranthene; n, Benzo[g,h,i]perylene; and o, Dibenz[a,h]anthracene.

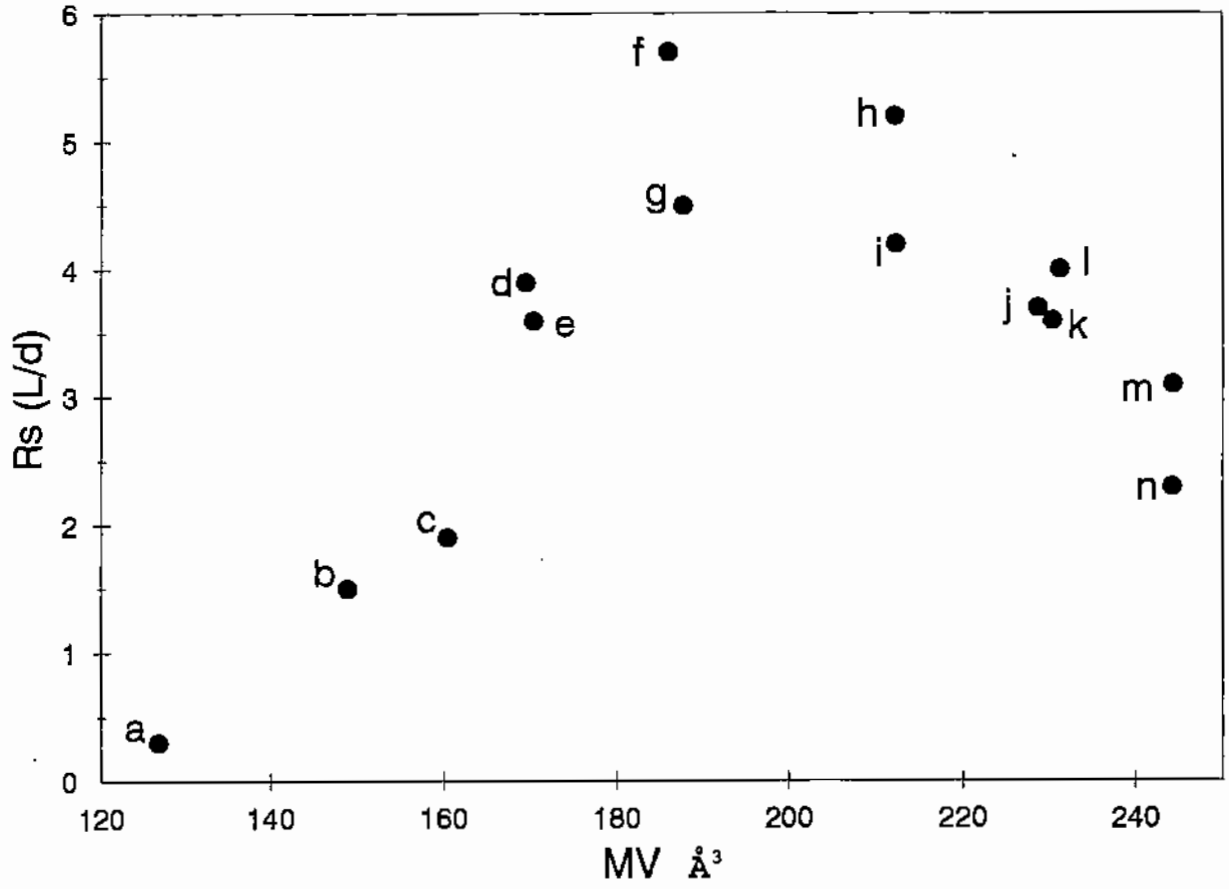

Figure 4. The potential relationship between SPMD sampling rates(Rs, 1 g triolein configuration) and PAH molecular volume (MV) in Å³. Data used are recovery corrected and from the 100 ng/L flow-through exposure (18°C) at 14 days. Each Rs value represents mean of three determinations (coefficients of variation <14% for all compounds except naphthalene [46%]) and analytes are designated by letters as follows: a, Naphthalene; b, Acenaphtene; c, Fluorene; d, Phenanthrene; e, Anthracene; f, Pyrene; g, Fluoranthene; h, Chrysene; i, Benz[a]anthracene; j, Benzo[a]pyrene; k, Benzo[b]fluoranthene; l, Benzo[k]fluoranthene; m, Dibenzo[a,h]anthracene; and n, Benzo[g,h,i]perylene.

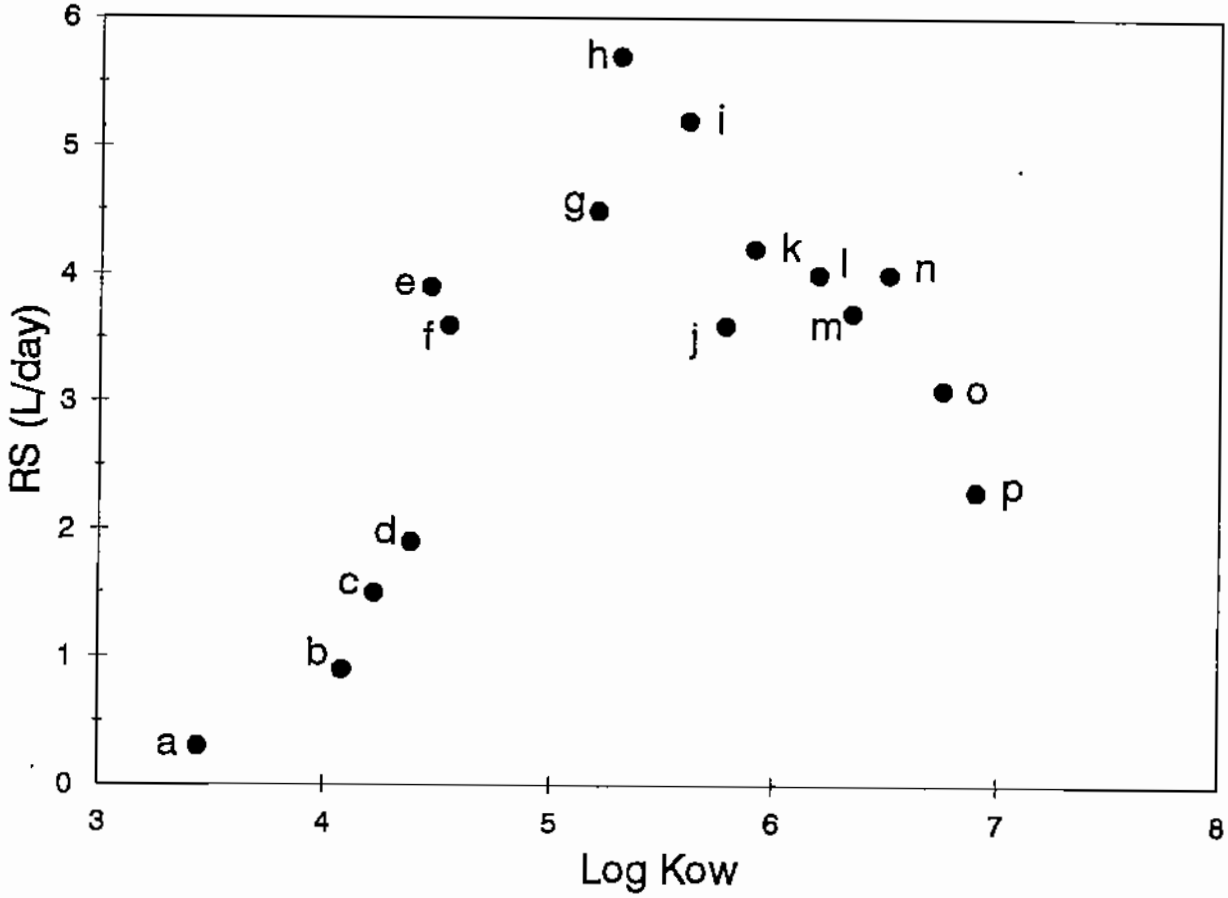

Figure 5. The potential relationship between SPMD sampling rates (Rs, 1 g triolein configuration) and PAH octanol-water partition coefficients. Data used are recovery corrected and from the 100 ng/L flow-through exposure (18°C) at 14 days. Each Rs value represents mean of three determinations (coefficients of variation <14% for all compounds except naphthalene [46%]). Analytes are designated by letters as follows: a, naphthalene; b, acenaphthylene; c, acenaphthene; d, fluorene; e, phenanthrene; f, anthracene; g, fluoranthene; h, pyrene; i, chrysene; j, benzo[b]fluoranthene; k, benz[a]anthracene; l, benzo[k]fluoranthene; m, benzo[a]pyrene; n, indeno[1,2,3-cd]pyrene; o, dibenz[a,h]anthracene; and p, benzo[g,h,i]perylene.

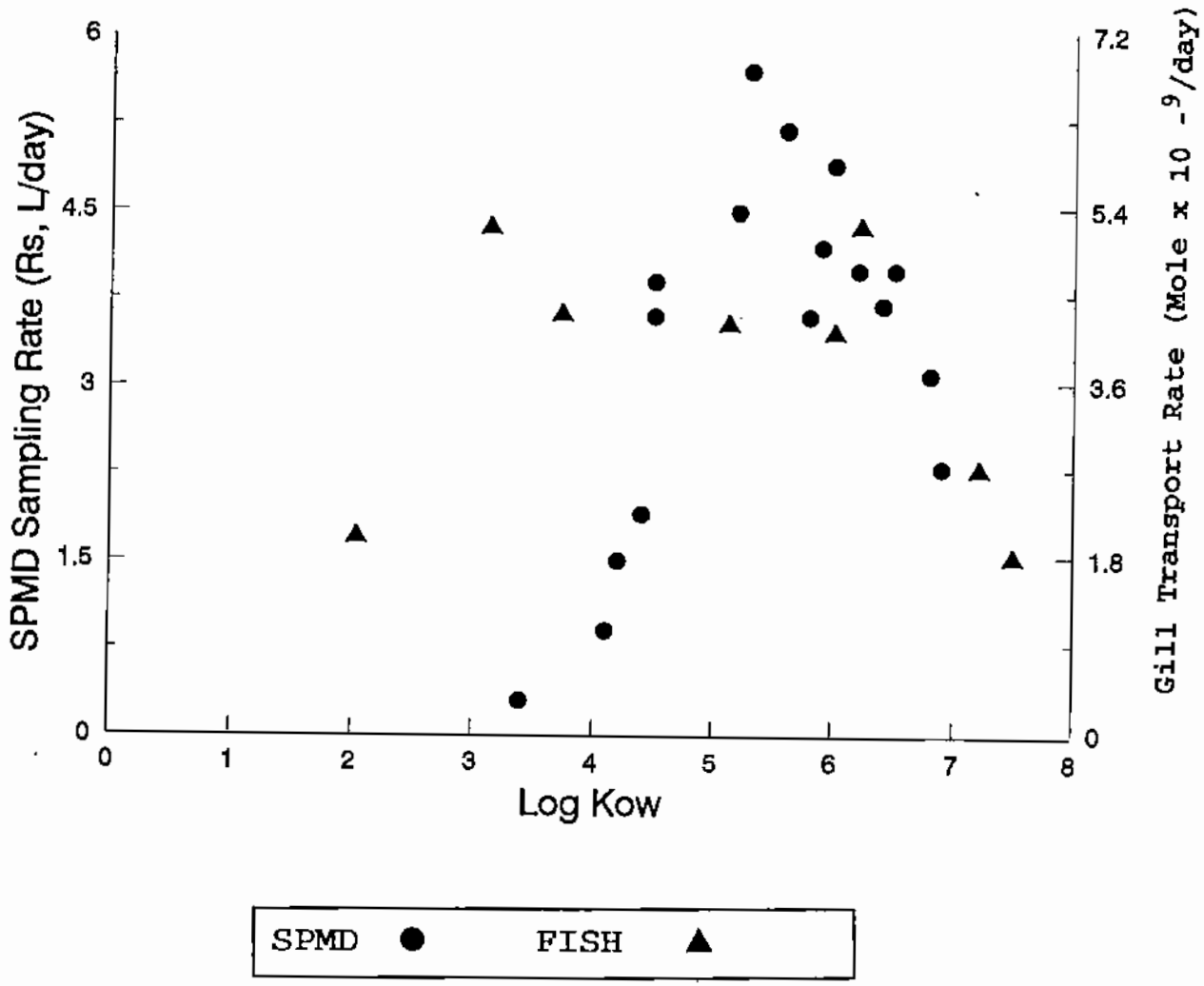

Figure 6. Comparison of PAH uptake (2,2',5,5-tetrachlorobiphenyl also included and denoted by circled value) by SPMDs (recovery corrected, 1 g triolein configuration, 14 day, 18°C, 100 ng/L) to the uptake of organic chemicals across the gill (circled value represents 2,2',5,5'-tetrachlobiphenyl) of fish (from McKinn et. al. 1985).

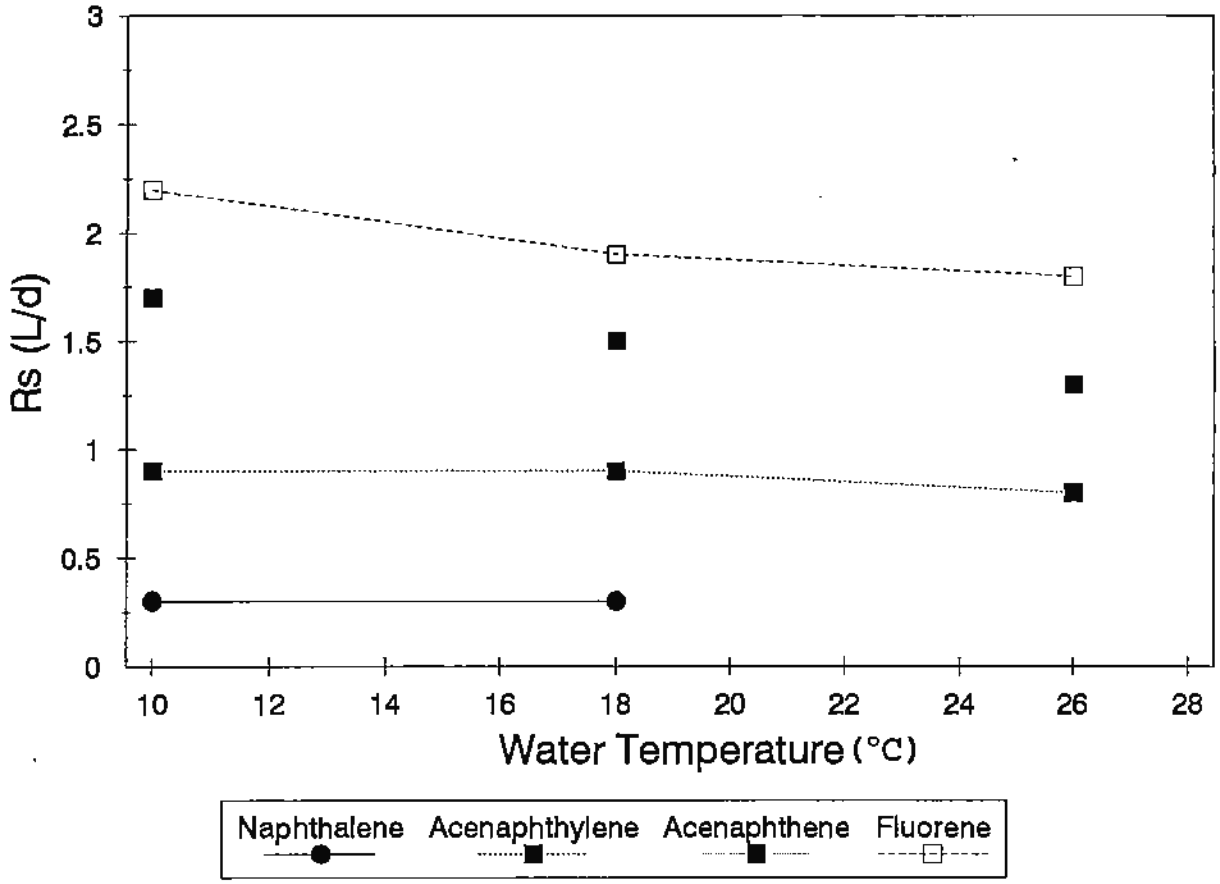

Figure 7. Effect of water temperature on SPMD sampling rates (Rs, 1 g triolein configuration) for PAHs. Values (flow-through, 100 ng/L, 14 day) are analytical recovery corrected and represent the mean of three determinations. In all cases (except naphthalene at 18°C [C.V.=46%]) the coefficients of variation are $\leq$ 10%.

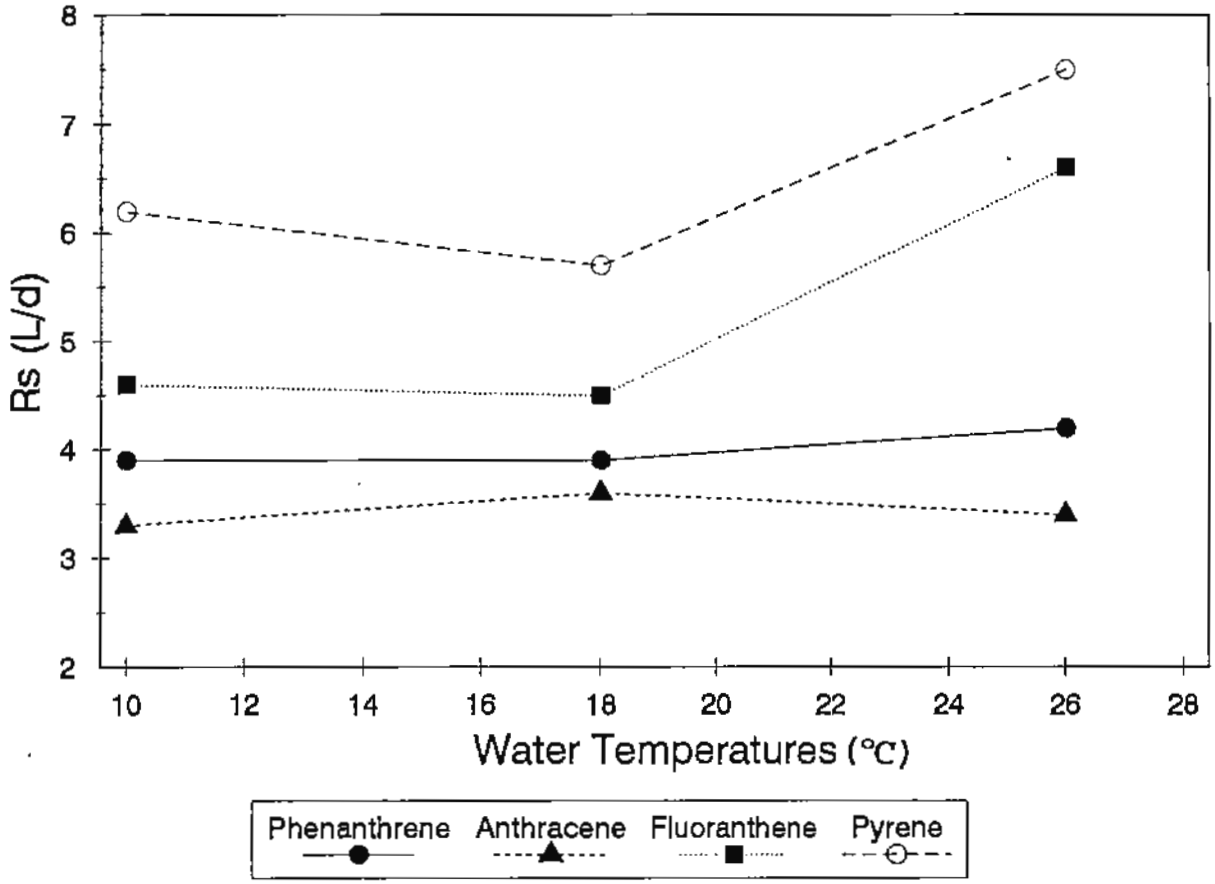

Figure 8. Effect of water temperature on SPMD sampling rates (Rs, 1 g triolein configuration) for PAHS. Values (flow-through, 100 ng/L, 14 day) are recovery corrected and represent the mean of three determinations. In all cases the coefficients of variation are ≤ 13%.

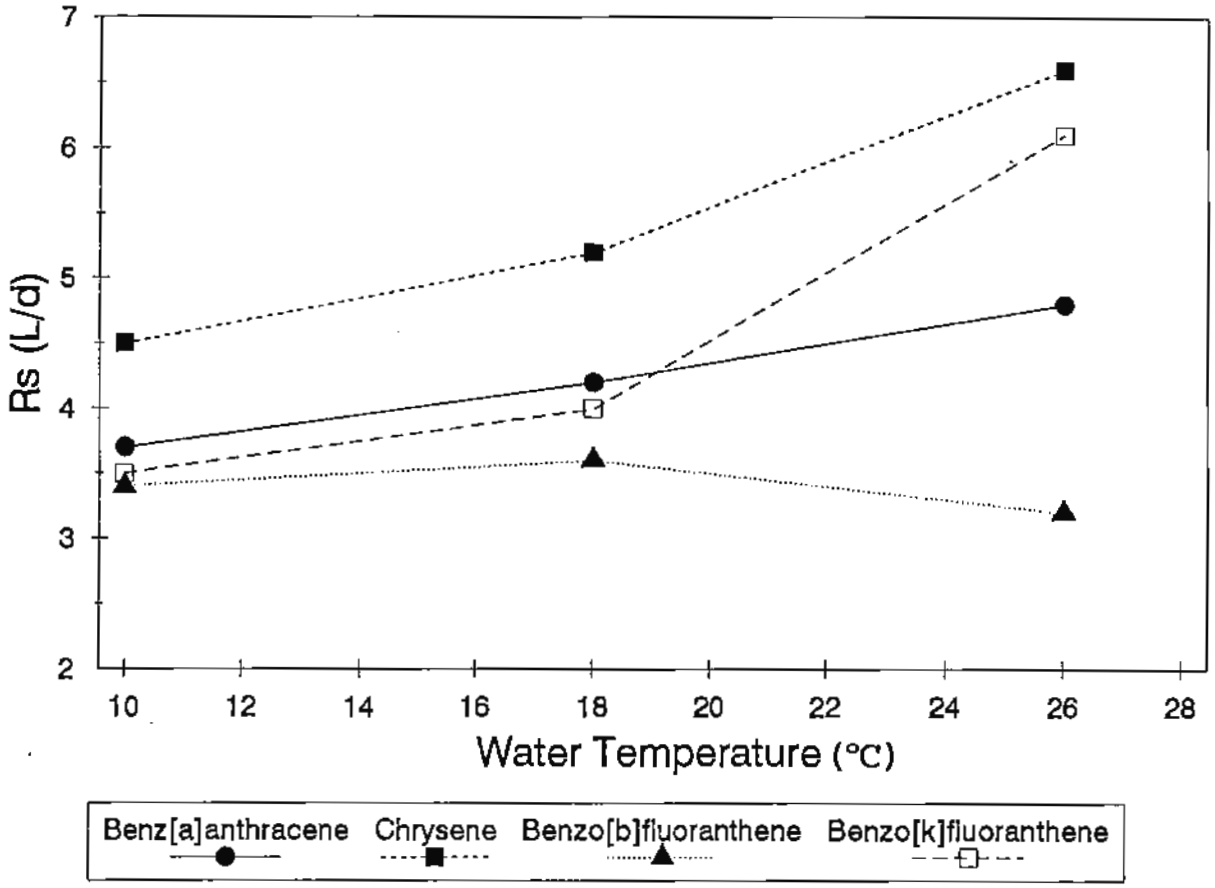

Figure 9. Effect of water temperature on SPMD sampling rates (Rs, 1 g triolein configuration) for PAHs. Values (flow-through, 100 ng/L, 14 day) are recovery corrected and represent the mean of three determinations. In all cases (except benzo[b]fluoranthene [20%]) the coefficients of variation are ≤ 12%.

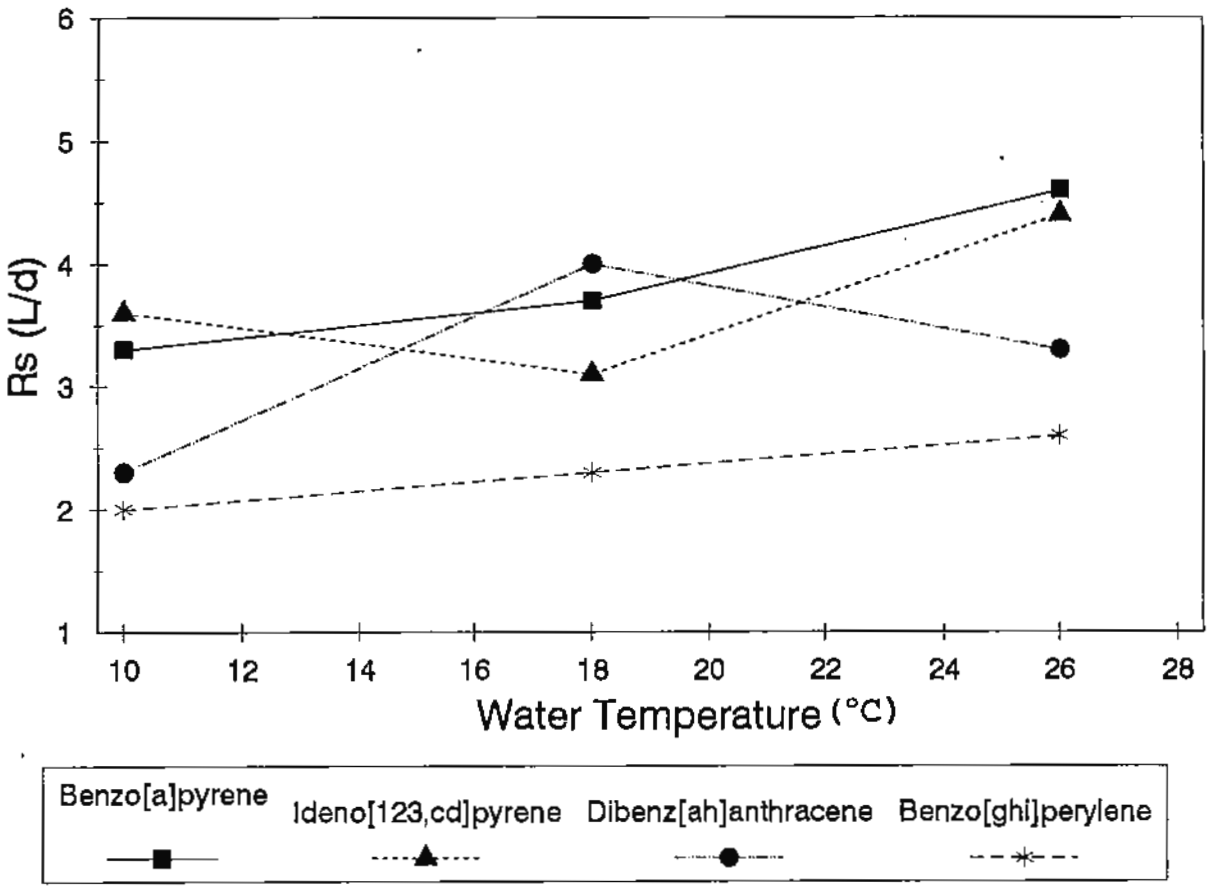

Figure 10. Effect of water temperature on SPMD sampling rates (Rs, 1 g triolein configuration) for PAHs. Values (flow-through, 100 ng/L, 14 day) are recovery corrected and represent the mean of three determinations. In all cases the coefficients of variation are ≤ 11%.

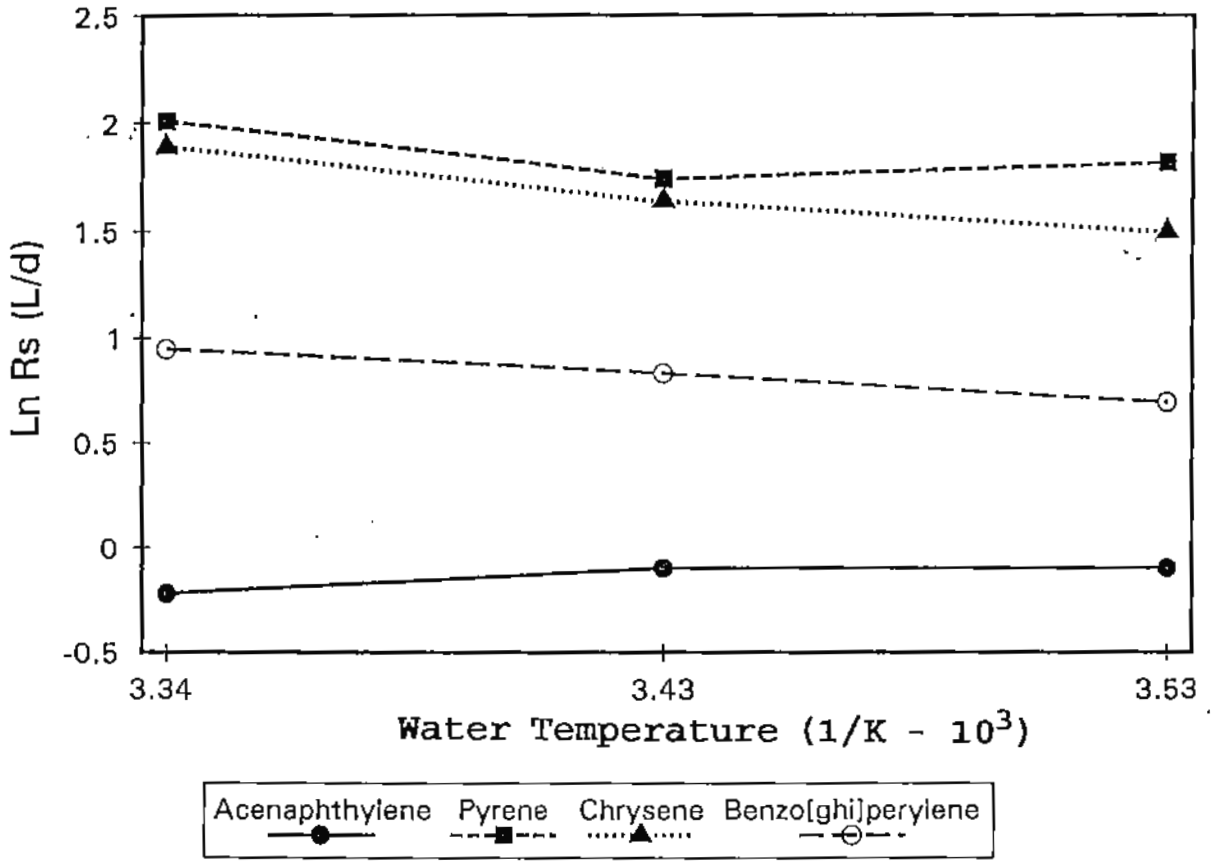

Figure 11. Arrhenius plot of the effect of water tempature on SPMD sampling rates (Rs, 1 g triolein configuration) for selected PAHs. Values (flow-though, 100 ng/L, 14 day) are recovery corrected and represent the mean of three determinations. In all cases the coefficients of variation are ≤ 12%.

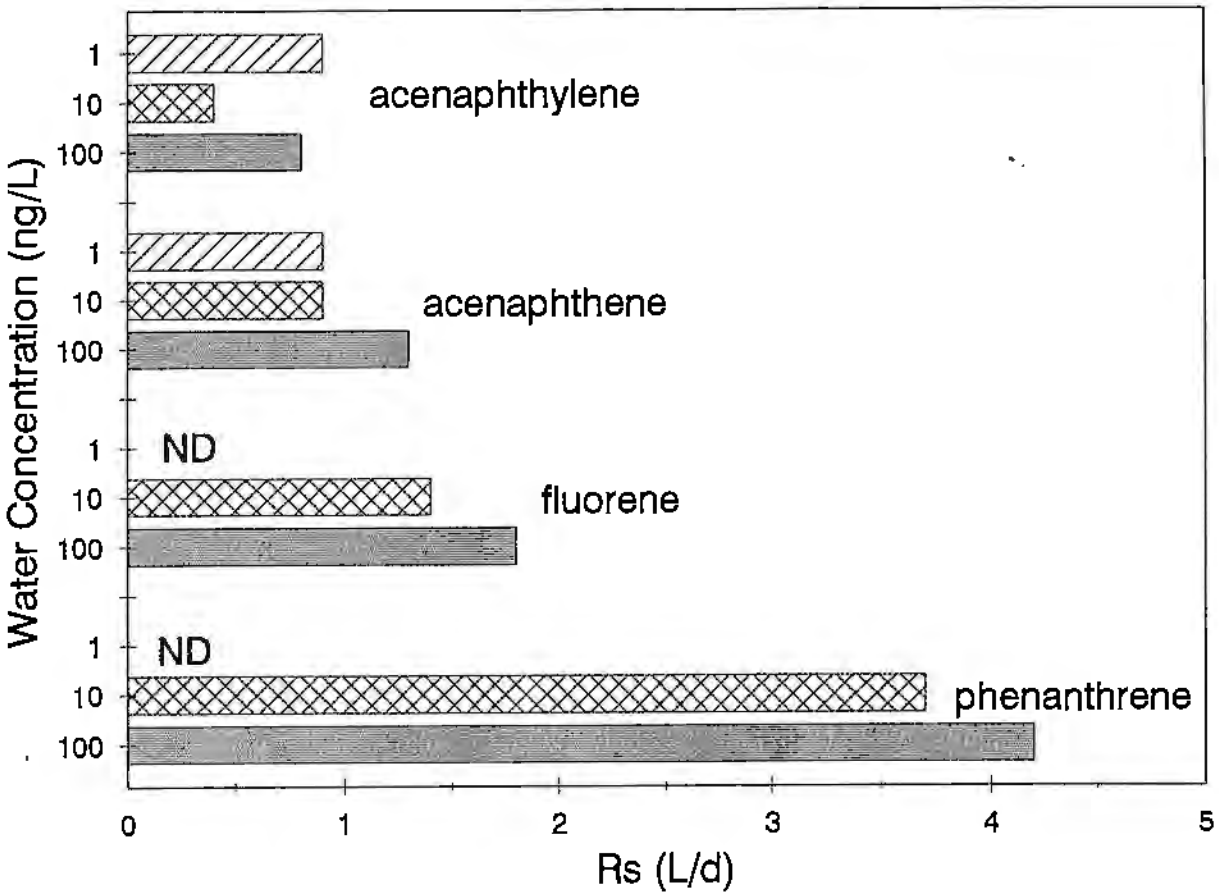

Figure 12. Effects of flow-through water concentration on SPMD sampling rates (Rs, 1 g triolein configuration) for PAHs. Data (14d, 26°C) are recovery corrected and represent the mean of three determinations (except acenaphthene, N=2). The coefficient variation are ≤ 9% for all 10 and 100 ng/L values. ND is none detected, see text for definition of detection limits.

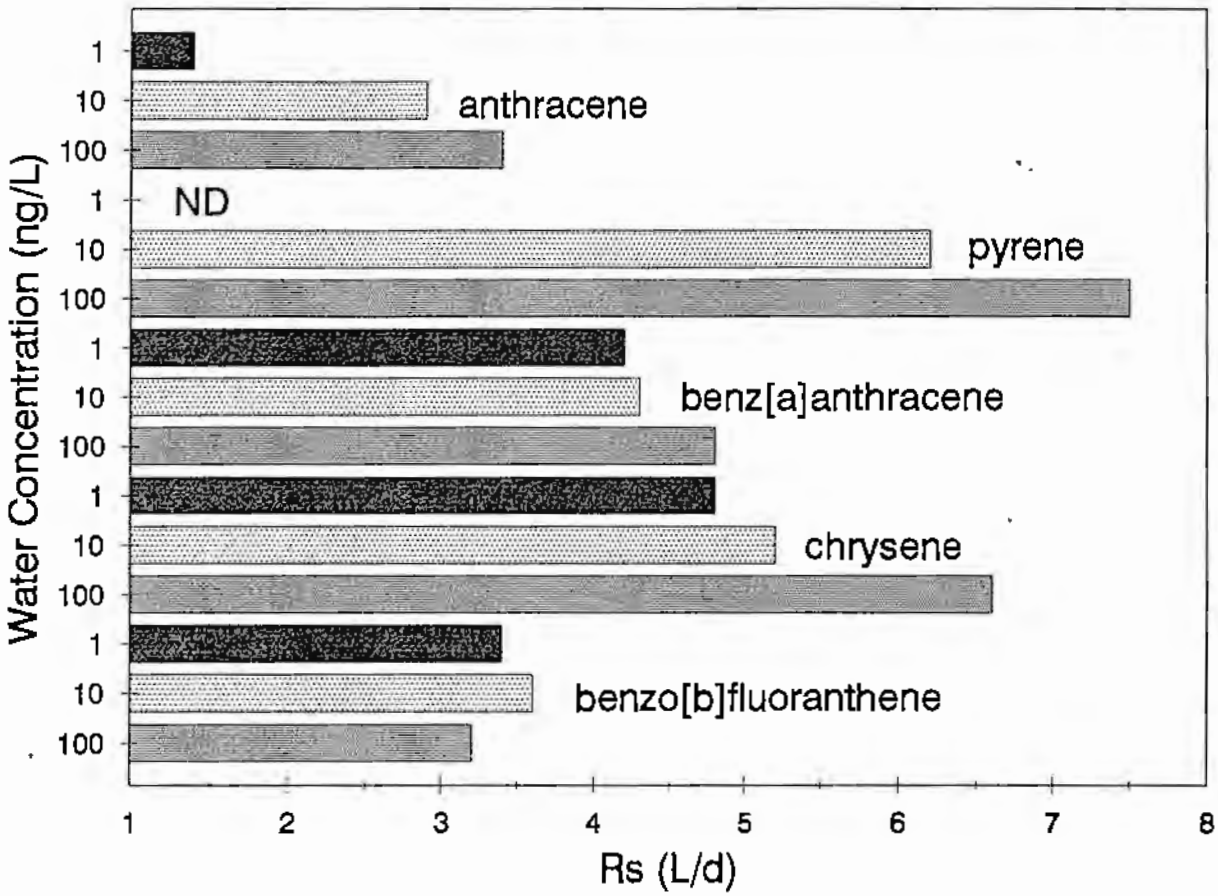

Figure 13. Effects of flow-through water concentration on SPMD sampling rates (Rs, 1 g triolein configuration) for PAHs. Data (14d, 26°C) are recovery corrected and represent the mean of three determinations. The coefficients of variation are ≤ 12% for 10 and 100 ng/L values and ≤ 47% for 1 ng/L values.

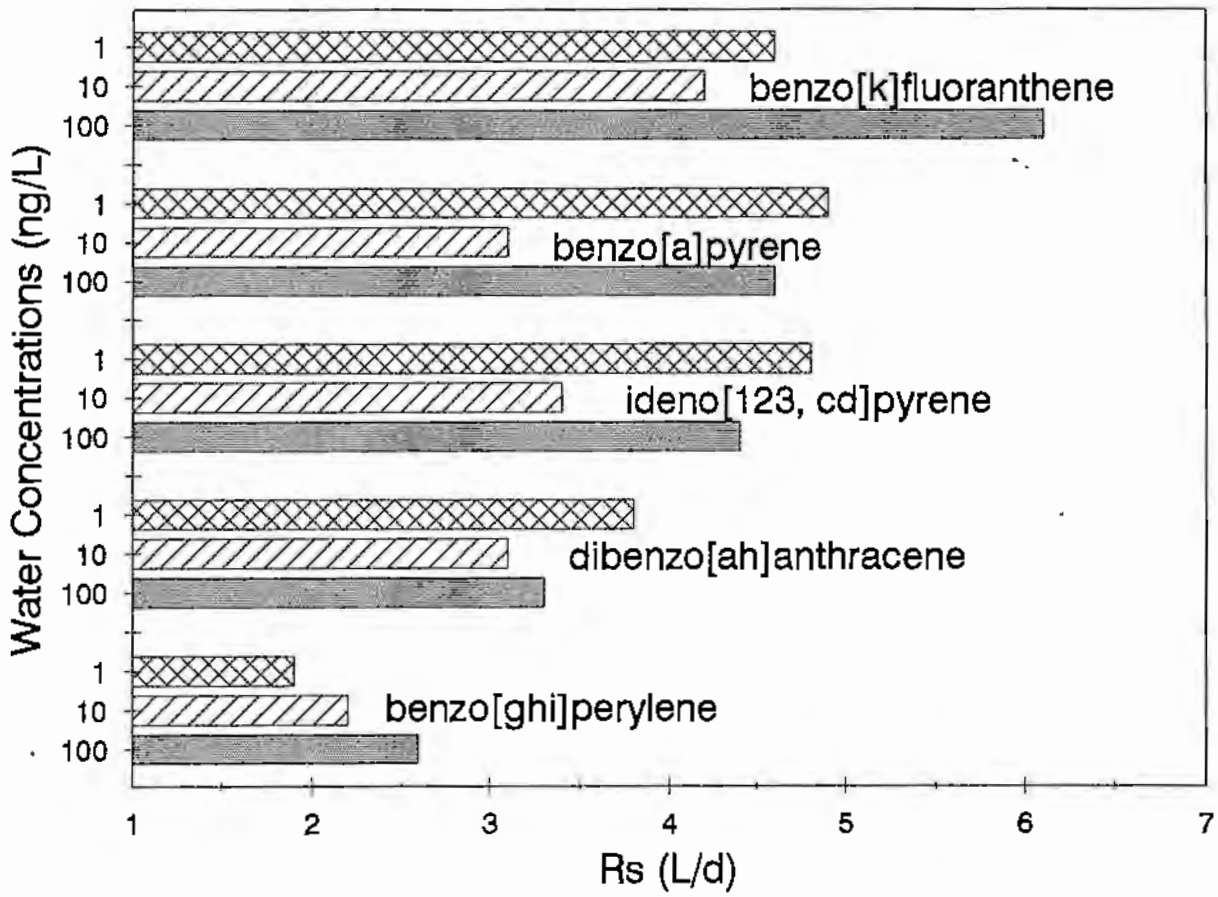

Figure 14. Effect of flow-through water concentration on SPMD sampling rates (Rs, 1 g triolein configuration) for PAHs. Data (14d, 26°C) are recovery corrected and represent the mean of three determinations (except anthracene and chrysene). The coefficients of variation are ≤ 20% for all 10 and 100 ng/L values. ND is none detected; see text for definition of detection limits.

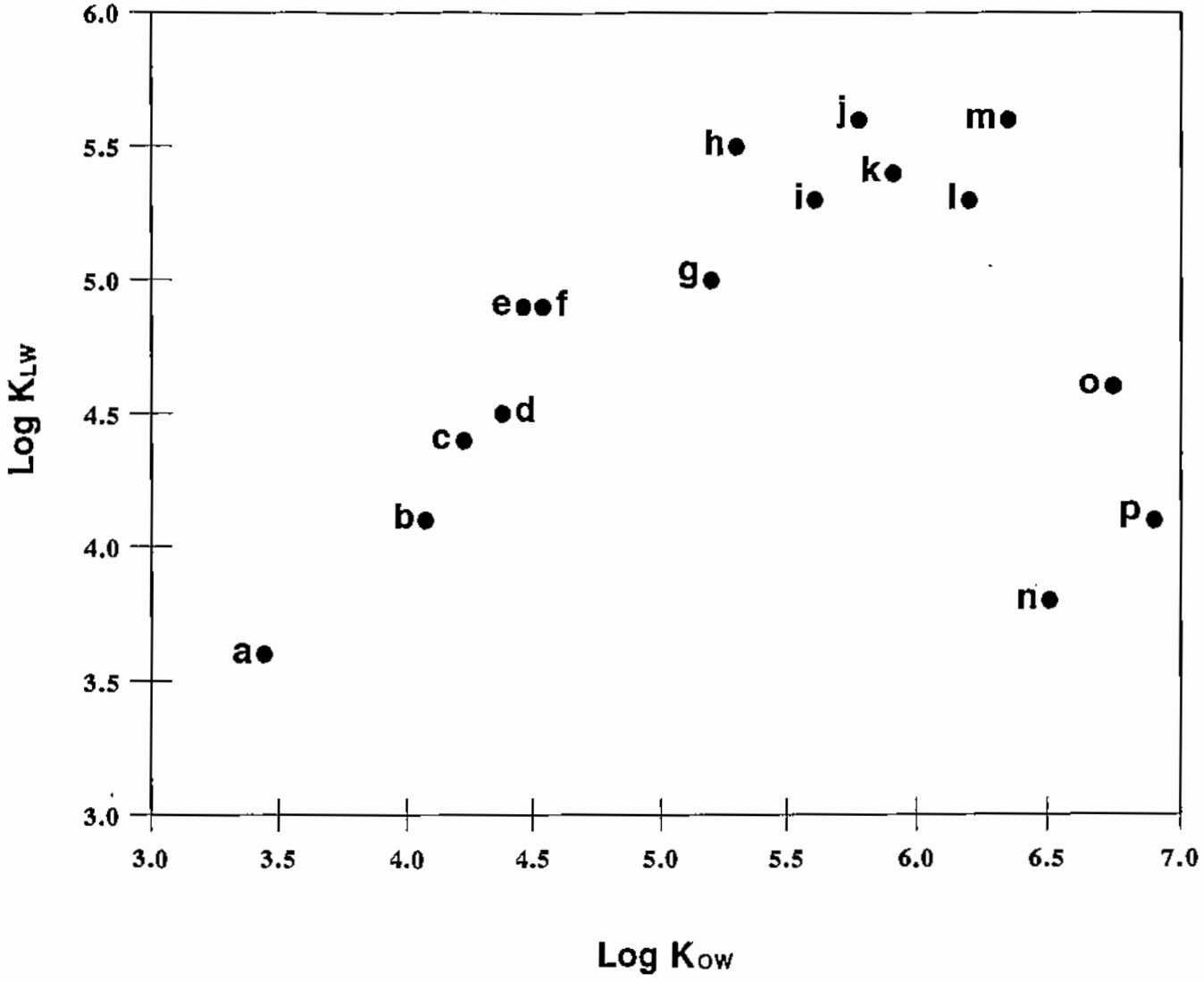

Figure 15. Potential relationship between log K_{OW}s and log K_{LW}s (28 day) for PAHs. Values are from single application freshwater static exposures. Letters represent: a, naphthalene; b, acenaphthylene; c, acenaphthene; d, fluorene; e, phenanthrene; f, anthracene; g, fluoranthene; h, pyrene; i, chrysene; j, benzo[b]fluoranthene; k, benz[a]anthracene; l, benzo[k]fluoranthene; m, benzo[a]pyrene; n, dibenz[ah]anthracene; o, indeno[123cd]pyrene; p, benzo[ghi]perylene.

APPENDIX A:

STUDY PROTOCOLS

NFCRC Protocol P-93-33-02

Work Unit 30078

Date Prepared: June 16, 1993

Study Title: Uptake of Priority Pollutant Polyaromatic Hydrocarbons (PAHs) by Semipermeable Membrane Devices (SPMDs) During Flow-through Aqueous Exposures at Various Concentrations and Temperatures.

This study is designed to characterize the uptake of 16 priority pollutant polyaromatic hydrocarbons (PAHs) by SPMDs under various concentrations and temperature conditions in flow-through laboratory exposures.

This research will be conducted in basic accordance with Good Laboratory Practices outlined in the Federal Register (160.120; 40 CFR Ch 1; 7-1-85 edition; subpart G - "Protocol for and conduct of a study:).

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1. Title and purpose of study.

Study Title: **Uptake of Priority Pollutant Polyaromatic Hydrocarbons (PAHs) by Semipermeable Membrane Devices (SPMDs) During Flow-through Aqueous Exposures at Various Concentrations and Temperatures.**

An understanding of the effects of temperature and contaminant concentration on the uptake of priority pollutant PAHs by SPMDs is essential to the estimation of ambient PAH water concentrations from SPMD data.

2. Identification of the test and control substances.

Test Toxicants:

2,000 ug/mL of each of the following priority pollutant PAHs obtained from Supelco, Inc:

Acenaphthene	Chrysene
Acenaphthylene	Dibenz(a,h)anthracene
Anthracene	Fluoranthene
Benz(a)anthracene	Fluorene
Benzo(a)pyrene	Indeno(1,2,3-cd)pyrene
Benzo(b)fluoranthene	Naphthalene
Benzo(g,h,i)perylene	Phenanthrene
Benzo(k)fluoranthene	Pyrene

Test Substances -

1. Triolein - High purity ($\geq$ 95%), Sigma Chemical Co.

2. Acetone - HPLC Grade, Fisher Chemical Co.

3. Sanaqua - Didecyl dimethyl ammonium chloride solution (7.5%), AquaVet Co.

4. Polyethylene layflat tubing - Virgin plastic, no additives. 0.0089 cm thick and 2.54 cm wide, Brentwood Plastics.

3. Name and address of sponsor and facility.

The sponsors for this study are the National Fish and Wildlife Foundation, Chevron Inc., AMOCO Inc., and NFCRC. The testing facility is the National Fisheries Contaminant Research Center (NFCRC), U.S. Fish and Wildlife Service, 4200 New Haven Rd., Columbia, MO. 65201.

4. Proposed starting and completion dates.

The study will begin in July 1993 and is anticipated to be completed by February 1994. The completion date relates to the termination of flow-through exposures and does not reflect time for data analysis and manuscript generation. The three tasks will be conducted sequentially due to personnel and equipment limitations.

5. Justification for selection of test system.

The primary reason for the selection of a constant flow-through system is to directly elucidate key SPMD-contaminant interactions. Calculation of ambient aquatic concentrations of different PAHs by analysis of field-exposed SPMDs is problematic without the empirically derived uptake kinetic information generated by this flow-through exposure study.

6. Species number, body weight, sex, and source.

Not applicable.

7. Procedure for identification of test system.

SPMD exposures to PAHs will be conducted in the Biology East Wet Laboratory. Diluter #1 area will be used in the Study.

Four 30 Liter aquaria (test chambers 1,2,3, & 4) will be required for the three 21 day SPMD exposures. Test chamber 1 will have a nominal water concentration of 1 ng/L of each PAH. Test chamber 2 will have a nominal water concentration of 10 ng/L of each PAH. Test chamber 3 will have a nominal water concentration of 100 ng/L of each PAH. Test chamber 4 is a control and will have a nominal water concentration of 0 ng/L of each PAH.

8. Experimental design.

See outline (Section 19).

9. Diet, solvents.

Test toxicants will be dissolved in nanograde acetone to prepare the Diluter Stock Solutions. 100 μL volumes of the Diluter Stock Solutions will be added to each liter of well water, via micromedic pumps, during a diluter dump cycle (10 minutes).

10. Route of administration.

The SPMDs will be suspended in the water column of the test chambers of the Diluter. Well water (at various controlled temperatures) will flow through the test chambers for 21 days.

The concentration of most PAHs in the test water will be at least an order of magnitude less than their water solubility ($\geq$ 1 μg/L).

11. Dosage level of control substances.

See Section 19 (Outline).

12. Method to measure degree of uptake.

Chemical residue analysis will be conducted for 16 PAHs in samples of SPMDs, test chamber water and Diluter Stock Solutions using the current versions of SOPs C5.139, C5.173, and C5.185.

13. Type and frequency of tests, analyses, and measurements.

See Section 19.

14. Records.

Records to be maintained include diluter calibration and operation, Diluter Stock calculations and measurements, diluter water temperature, and analysis of PAH concentrations in diluter test chamber water and SPMDs.

Until archiving, data will be stored on data sheets and in laboratory notebooks located in the offices of R. Clark, G. Gibson, J. Lebo, C. Orazio, J. Huckins, and J. Petty. Also data will be stored in the NIC 486 computer in room 15. Permanent archiving of data will follow SOP B5.147.

15. Date of approval of protocol.

See Section 17.

16. Statistics.

Data from these experiments will be entered into Lotus 1-2-3 spreadsheet files for the generation of spreadsheets and limited statistics such as test compound concentrations, means and Standard Deviations.

Further analyses will be conducted using Harvard Graphics or SigmaPlot Scientific Graph System or other computer software.

17. Signatures.

Prepared by: Randal Clark
Environmental Chemistry

Approved by: James Huckins
Research Chemist

Jim Petty
Research Scientist

7/2/93
Ted Schwartz
Chief Chemist

for C Ingersoll 7/2/93
Chris Ingersoll
Fisheries Biologist

7-6-93
Joseph B. Hunn
Quality Assurance Officer

7-6-93
Richard A. Schoettger
Center Director

18. SOPs used in Study.

SOP #	Date	Title
B5.106	11/28/88	Safety Plan for the Fish and Invertebrate Toxicology Section.
B5.147	09/14/88	Permanent Archiving of Handwritten Materials.
B5.217	03/03/90	Preparation of Semipermeable Polymeric Membrane Devices (SPMDs) with Model Lipid for Monitoring Organic Contaminants in Water.
C5.72	11/05/87	The Cleaning of Labware for Use in Organic Contaminant Residue Analysis.
C5.139	9/28/90	Gas Chromatographic Procedure or Screening and Semi-quantitative Analysis of Sample Solutions Prior to Determinative Analysis.
C5.173	01/06/92	Semipermeable Membrane Device (SPMD) Cleanup and Preparation for Analysis.
C5.185	06/25/92	Procedure for the Extraction, Cleanup, and Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediment and Soil Samples.

19. Outline of PAH Diluter Study.

I. **Test Schedules -**

A. Task A - 10 °C Diluter test conducted in summer 1993.

B. Task B - 18 °C Diluter test conducted in fall 1993.

C. Task C - 26 °C Diluter test conducted in winter 1993.

All three of the above tasks are considered one study and are identical with the exception of the diluter temperature.

II. **Test Toxicants -**

A. 2,000 ug/mL of each of the following priority pollutant PAHs obtained from Supelco, Inc:

Acenaphthene	Chrysene
Acenaphthylene	Dibenz(a,h)anthracene
Anthracene	Fluoranthene
Benz(a)anthracene	Fluorene
Benzo(a)pyrene	Indeno(1,2,3-cd)pyrene
Benzo(b)fluoranthene	Naphthalene
Benzo(g,h,i)perylene	Phenanthrene
Benzo(k)fluoranthene	Pyrene

III. **Test Substances -**

A. Triolein - High purity ($\geq$ 95%), Sigma Chemical Co.

B. Acetone - HPLC Grade, purchased from Fisher Chemical Co.

C. Sanaqua - Didecyl dimethyl ammonium chloride solution (7.5%), purchased from AquaVet Co.

D. Polyethylene layflat tubing - Virgin plastic, no additives. $\approx$ 0.0089 cm thick and 2.54 cm wide, purchased from Brentwood Plastics.

IV. **Test Parameters -**

A. **Test Location -**

1. Biology East Wet Lab.

2. Flow-through Diluter # 1.

B. **Test Temperatures -**

1. Task 1, 10 °C diluter water -

   Diluter well water will need to be chilled from 18 °C to 10 °C.

2. Task 2, 18 °C diluter water -

   Diluter well water average temperature is 18 °C. No alteration of temperature of diluter water is needed.

3. Task 3, 26 °C diluter water -

   Diluter well water will need to be heated from 18 °C to 26 °C.

C. **Test volume** - 30 L per test chamber (aquaria).

D. **Test Water -** NFCRC well water (hardness = 294 mg/L as $CaCO_3$, total alkalinity = 256 mg/L as $CaCO_3$, DOC = 1.84 mg/L, POC = 0.87 mg/L, and TOC = 2.70 mg/L).

E. **Test light cycle** - Normal lab light cycle. The test chambers will be covered to minimize possible photolysis of the PAHs.

F. **Test Chamber turnover rate -** Diluter dump cycle consists of ≈1 L per 10 minutes, resulting in a turnover rate of ≈5 hours per 30 L test chamber.

G. **Test length** - 21 days per task.

H. **Test Subjects -**

1. Semipermeable Membrane Device (SPMD) = 2.54 cm wide polyethylene layflat tubes that are 45.7 cm long (pre-extracted with hexane), containing 0.42 g (0.5 mL) triolein configured as a thin film.

2. A total of 165 SPMDs will be used in the three tasks.

I. **Test Concentrations -**

1. **Control test chamber -**

   a. 100 μL of acetone in 1 L well water will be delivered to the 30 L control test chamber (#4) every dump cycle (10 minutes).

b. Solvent concentration in the control test chamber will be ≈105 μL/L. ≈370 mL of acetone will be used in the control test chamber (#4) during each task.

2. **Low concentration test chamber -**

a. A 2,000 mL solution containing each of the 16 PAH toxicants at a concentration of ≈10 μg/L in acetone will be prepared. This will be the low concentration Diluter Stock.

b. 100 μL of low concentration Diluter Stock will be delivered to the 30 L low concentration test chamber (#1) every dump cycle (10 minutes).

c. This will provide a 1 ng/L nominal concentration of each PAH to the low concentration test chamber.

d. Solvent (acetone) concentration in the low concentration water will be 100 μL/L.

3. **Medium concentration test chamber -**

a. A 2,000 mL solution containing each of the 16 PAH test toxicants at a concentration of ≈100 μg/L in acetone will be prepared. This will be the medium concentration Diluter Stock.

b. 100 μL of medium concentration Diluter Stock will be delivered to the 30 L medium concentration test chamber (#2) every dump cycle (10 minutes).

c. This will provide a 10 ng/L nominal concentration of each PAH to the medium concentration test chamber.

d. Solvent (acetone) concentration in the medium concentration water will be 100 μL/L.

4. **High concentration test chamber -**

a. A 2,000 mL solution containing each of the 16 PAH test toxicants at a concentration of ≈1000 μg/L in acetone will be prepared. This will be the high concentration Diluter Stock.

b. 100 μL of high concentration Diluter Stock will be delivered to the 30 L high concentration test chamber (#3) every dump cycle (10 minutes).

c. This will provide a 100 ng/L nominal concentration of each PAH to the low concentration test chamber.

d. Solvent (acetone) concentration in the high concentration water will be 100 μL/L.

J. **Diluter setup, Day 0 -**

1. **SPMDs holding ring -**

a. A 1 foot diameter stainless steel ring will be suspended under the center of the diluter water inlet spout for each test chamber (1-4) (N=4).

b. Each of the rings of test chamber 1-3 will be divided into three quadrants using aluminum wire. Each quadrant having a 120° arc.

c. The ring of test chamber 4 will be divided into 4 quadrants using aluminum wire. Each quadrant having a 90° arc.

2. **SPMDs placed in diluter test chambers on Day 0 -**

a. Three SPMDs will be prepared on day 0. These SPMDs will be blanks used to determine the PAH background of the SPMDs (N=3 per task, N=9 per study).

b. **Test chamber 1 -**

(1) For each Task, 12 SPMDs will be placed in test chamber 1. 4 SPMDs will be in each quadrant (3) of the SPMD holding ring (N=12 per task, 36 per study).

(2) SPMDs in test chamber 1 will be used for the low treatment or concentration.

c. **Test chamber 2 -**

(1) For each Task, 12 SPMDs will be placed in test chamber 2. 4 SPMDs will be in each quadrant (3) of the SPMD holding ring (N=12 per task, 36 per study).

(2) SPMDs in test chamber 2 will be used for the medium treatment or concentration.

d. **Test chamber 3 -**

(1) For each Task, 12 SPMDs will be placed in test chamber 3. 4 SPMDs will be in each quadrant (3) of the SPMD holding ring (N=12 per task, 36 per study).

(2) SPMDs in test chamber 3 will be used for the high treatment or concentration.

e. **Test chamber 4 -**

(1) For each Task, 16 SPMDs will be placed in test chamber 4. 4 SPMDs will be in each quadrant (4) of the SPMD holding ring (N=16 per task, 48 per study).

(2) SPMDs in test chamber 4 will be used for the control treatment or concentration.

f. **Total SPMDs required for Study.**

(1) Blank SPMDs - (N=3 per task, N=9 per study).

(2) Low Concentration SPMDs - Used in test chamber 1 (N=12 per task, N=36 per study).

(3) Medium Concentration SPMDs - Used in test chamber 2 (N=12 per task, N=36 per study).

(4) High Concentration SPMDs - Used in test chamber 3 (N=12 per task, N=36 per study).

(5) Control Concentration SPMDs - Used in test chamber 4 (N=16 per task, N=48 per study).

(6) Total SPMDs placed in Diluter on Day 0 N=52 per task, 156 per study.

(7) Total SPMDs required for the Study N=56 per task, 165 per study.

K. **Treatment of SPMDs -**

1. SPMDs in all a test chamber in Diluter 1 will be dipped in Sanaqua on Study Days 0, 7 and 14 to retard biological growth on outside surface.

V. **Test measurements -**

A. **Sampling Method -**

1. **SPMDs -**

a. SPMDs will be collected on Study Days 4, 7, 14, and 21 (4 sampling times) for each test chamber (1-4).

b. Collected SPMDs will be place in solvent rinsed Aluminum foil and frozen (-25 °C) until analysis.

2. **Test chamber Water -**

a. A 500 mL glass beaker will be submerged in each test chamber throughout the study in order to equilibrate with test chamber water. This beaker will be used to sample the Diluter water.

b. Water will be collected in brown glass 4,000 mL bottles (teflon lined lid) with 100 mL methylene chloride added as keeper to each bottle.

c. Water samples will be kept in refrigerator (5 °C) until analysis.

B. **Sampling schedule for PAH analysis -**

1. **SPMDs -**

a. **Sampling schedule for SPMDs on Day 0 -**

On Day 0, three replicate Blank SPMDs will be prepared for analysis (N=3 per task, N=9 per study).

b. **Sampling Schedule for SPMDs in Diluter (Days 4, 7, 14, and 21) -**

One SPMDs will be collected from each quadrant of the SPMD ring of each test chamber on sample days 4, 7, 14, and 21 (N=3 in test chamber 1-3 and 4 in test chamber 4, N=13 per Diluter per sample day, N=52 per task and N=156 per study).

2. **Test chamber Water Sampling -**

One water sample from each test chamber will be collected on study days 0 and 21.

One additional water sample will be randomly collected from one of the test chamber on study days 0 and 21 as a QC sample replicate.

a. **Test chamber 1 (low concentration) -**

Collect 8 L of test chamber water by placing 2 L of water in four 4 L bottles.

b. **Test chamber 2 (medium concentration) -**

Collect 4 L of test chamber water by placing 2 L of water in two 4 L bottle.

c. **Test chamber 3 (high concentration) -**

Collect 2 L of test chamber water by placing 2 L of water in a 4 L bottle.

d. **Test chamber 4 (control concentration) -**

Collect 8 L of test chamber water by placing 2 L of water in four 4 L bottles.

e. **Water Spike**

Collect 8 L of water from test chamber 4 and spike with appropriate amount of Diluter Stock.

f. **Total water samples collected**

6 per sample day, 12 per task, 36 per study.

3. **Diluter Stock Solutions -**

   a. One 2 mL sample from each of the Diluter Stock Solutions (N=4) will be collected on Day 0 (N=4 per Task, N=12 per Study).

   b. One 2 mL sample from each of the Diluter Stock Solutions (N=4) will be collected on Day 21 (N=4 per Task, N=12 per Study).

   c. Total Diluter Stock Solutions collected will be N=8 per Task, N=24 per Study).

4. **Lagoon Effluent Water Samples -**

   a. 1 water sample (1 L) will be collected from NFCRC's lagoon effluent before the task begins. (N=1 per Task, 3 per Study).

   b. 1 water sample (1 L) will be collected from NFCRC's lagoon effluent during each task (N=1 per Task, 3 per study).

   c. Total lagoon effluent samples collected will be N=2 per Task, 6 per study.

5. **Total Diluter Samples collected in Diluter Study.**

   a. Blank SPMDs - 3 per Task, 9 per Study.

   b. Diluter SPMDs - 52 per Task, 156 per Study.

   c. Diluter Water - 12 per Task, 36 per Study.

   d. Diluter Stock Solutions - 8 per task, 24 per Study.

   e. Lagoon Effluent - 2 per Task, 6 per Study.

   f. Total Samples Analyzed - 77 per Task, 231 per Study.

C. **Method of chemical measurement -**

1. Chemical residue analysis for PAHs in all diluter samples, which includes stock solutions, SPMDs, and test chamber water.

   a. Test Chamber Water Samples -

      Using current version of SOP C5.185.

b. SPMD Membrane Samples -

Using current version of SOP C5.185, and SOP C5.173.

c. All Other Diluter Samples -

Using current version of SOP C5.185.

2. **Test chamber water quality -**

Diluter temperature measured daily.

D. **Method of disposal of diluter effluent -**

Effluent from the test chambers of the Diluter will go into the Blueline pipe. The Blueline pipe goes to the treatment system for the removal of toxicants by activated charcoal before being released to the environment.

NFCRC Protocol: P-94-33-02

Work Unit No.: 30078

Date Prepared: December 20, 1993

STUDY TITLE: Concentration of Priority Pollutant Polyaromatic Hydrocarbons (PAHs) by Semipermeable Membrane Devices (SPMDs) in static aqueous exposure systems.

This study is designed to elucidate the equilibrium partition coefficients of 16 priority pollutant polyaromatic hydrocarbons (PAHs) in SPMD membrane and lipid.

This research will be conducted in general accordance with Good Laboratory Practices outlined in the Federal Register (160.120; 40 CFR Ch.1; 7-1-85 edition; subpart G- "Protocol for and conduct of a study").

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1. Title and Purpose of Study

Title of Study: Concentration of Priority Pollutant Polyaromatic Hydrocarbons (PAHs) by Semipermeable Membrane Devices (SPMDs) in static aqueous exposure systems.

Purpose of Study: A knowledge of priority pollutant PAH partition coefficients in SPMD components is essential to the estimation of ambient PAH water concentrations from SPMD data.

2. Identification of Test and Control Substances

Test Toxicants:

2,000 μg/mL of ampules each of the following priority pollutant PAHs were obtained from Supelco, Inc:

Acenaphthene
Acenaphthylene
Anthracene
Benz(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benz(g,h,i)perylene
Benzo(k)fluoranthene
Chrysene
Dibenz(a,h)anthracene
Flouranthene
Flourene
Indeno(1,2,3-cd)pyrene
Naphthalene
Phenanthrene
Pyrene

Test Substances:

1. Triolein - High purity (≥ 95%), Sigma Chemical Co.

2. Acetone - HPLC Grade, Fisher Chemical Co.

3. Polyethylene layflat tubing - Virgin plastic, no additives. Dimension of 0.0089 cm wall thickness and 2.54 cm wide, purchased from Brentwood Plastics.

3. Name and Address of Sponsor and Testing Facility

The sponsors for this study are the National Fish and Wildlife Foundation, Chevron Inc., AMOCO Inc., and NFCRC. The testing facility is the National Fisheries Contaminant

Research Center (NFCRC), U.S. NBS, 4200 Haven Road, Columbia, Missouri 65201.

4. **Proposed Starting and Completion Dates**

The study will begin in late January or early February 1994 and is anticipated to be completed by March 1994. The completion date relates to the termination of static exposures and does not reflect time for data analysis and possible manuscript generation.

5. **Justification for Selection of the Test System**

The primary reason for the selection of a static exposure microcosm system is to permit rapid achievement of key SPMD-contaminant equilibrium partition coefficients. Calculation of ambient aquatic concentrations of different PAHs by analysis of field-exposed SPMDs is problematic without the empirically derived partition coefficient information generated by this aqueous exposure study.

6. **Species Number, Body Weigh, Sex and Source**

Not applicable.

7. **Procedure for Identification of Test Systems**

SPMD exposures to PAHs will be conducted in the main laboratory, Room 18 chemistry lab.

Eighteen 1 L glass microcosm chambers (ball jars) will be required for this 56 day static exposure. Each chamber will contain 950 mL of NFCRC well water and one 0.1 ml triolein SPMD ($\simeq$ 4" long). After assembly the microcosms will be placed in a water bath at room temperature and each dosed with 1 μg of each (16) priority pollutant PAH.

8. **Experimental Design**

See outline (Section 19).

9. **Diet and Solvents**

Test chemicals will be dissolved in nanograde acetone to prepare the Stock Solution. A 100 μL volume of the Stock Solution will be added to well water in each microcosm.

10. **Route of Administration**

SPMDs will be suspended in the water columns of test chambers. Each chamber will receive a single dose (100 μl acetone) of the 16 PAHs using a Hamilton syringe (glass-

stainless steel).

11. **Dosage Levels of Control Substances**

See Section 19 (Outline).

12. **Method to Measure Degree of Uptake**

Chemical residue analysis will be conducted for the 16 PAHs in samples of SPMD lipid and membrane and test chamber water. The current versions of SOPs C5.139, C5.173, and C5.185 will be used for these analyses.

13. **Type and Frequency of Tests, Analyses, and Measurements**

See Section 19.

14. **Records**

Records to be maintained include the amount of PAHs spiked, water temperature, and analyses of PAH concentrations in test chamber water and SPMD components through time. Also QC sample data will be recorded.

15. **Date of Approval of Protocol**

See Section 17.

16. **Statistics**

Data from these experiments will be entered into Lotus 1-2-3 spreadsheet files for the generation of spreadsheets and limited statistics such as test compound concentrations, means and Standard Deviations.

Further analyses may be conducted using Harvard Graphics or SigmaPlot Scientific Graph System or other computer software.

17. **Signatures**

Prepared by: _____ 1-24-94
Jim Huckins — Date
Research Chemist

Approved by: _____ 1/24/94
Jim Petty — Date
Research Scientist

Ted Schwartz 1/24/94
Chief Chemist Date

Joe Hynn 1-24-94
Quality Assurance Officer Date

Richard A. Schoettger 1-24-94
Center Director Date

18. **SOPs Used in Study**

SOP #	DATE	TITLE
B5.147	09/14/88	Permanent Archiving of Handwritten Materials
B5.217	03/03/90	Preparation of Semipermeable Polymeric Membrane Devices (SPMDs) with Model Lipid for Monitoring Organic Contaminants in Water.
C5.72	11/05/87	The Cleaning of Labware for Use in Organic Contaminant Residue Analysis.
C5.139	09/28/90	Gas Chromatographic Procedure or Screening and Semi-quantitative Analysis of Sample Solutions Prior to Determinative Analysis.
C5.173	01/06/92	Semipermeable Membrane Device (SPMD) Cleanup and Preparation for Analysis.
C5.185	06/25/92	Procedure for the Extraction, Cleanup, and Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in Sediment and Soil Samples.

19. **Outline of PAH Static Exposure**

I. Test Schedule -

Exposure initiation will be in early January and the exposure completion date will be early March. Three replicate exposure chambers (SPMD and water will be sampled) on exposure days 1, 4, 7, 14, 28 and 56. Afterwards, samples will be processed and analyzed using PID-GC by early April. Date of final results is not yet established.

II. Test Toxicants -

The following priority pollutant PAHs were obtained from Supelco, Inc. and will be used in this study

Acenaphthene
Acenaphthylene
Anthracene
Benz(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benz(g,h,i)perylene
Benzo(k)fluoranthene
Chrysene
Dibenz(a,h)anthracene
Flouranthene
Flourene
Indeno(1,2,3-cd)pyrene
Naphthalene
Phenanthrene
Pyrene

III. Test Substances -

A. Triolein - High purity ($\geq$ 95%), Sigma Chemical Co.

B. Acetone - HPLC Grade, purchased from Fisher Chemical Co.

C. NFCRC well water (See IV D.)

D. Polyethylene layflat tubing - Virgin plastic, no additives. $\approx$ 0.0089 cm thick and 2.54 cm wide, purchased from Brentwood Plastics.

IV. Test Parameters -

A. Test Location

1. NFCRC, room 18, middle lab bay

B. Test Temperature

1. This test will be conducted at room temperature which average ≃ 25°C over a weekly period. A water bath for exposure chambers will reduce rapid temperature fluctuations and water temperatures will be routinely monitored.

C. Test Volume - 950 mL of water per test chamber (microcosm)

D. Test Water - NFCRC well water (hardness = 294 mg/L as $CaCO_3$, total alkalinity = 256 mg/L as $CaCO_3$, DOC = 1.84 mg/L, POC = 0.87 mg/L, and TOC = 2.70 mg/L).

E. Test Light Cycle - Normal lab light cycle. The test chambers will be covered to minimize possible photolysis of the PAHs.

F. Test Chamber Turnover Rate - N/A, single application static exposure.

G. Test Length - 56 days.

H. Test Subjects -

1. Semipermeable Membrane Device (SPMD) = 2.54 cm wide polyethylene layflat tubes that are 10.2 cm long (pre-extracted with hexane), containing 0.1 g (0.1 mL) triolein configured as a thin film.

2. A total of 18 SPMDs will be used in this exposure, plus 6 blanks (1 for each sample time).

3. Three components will be analyzed in each test chamber (subject) which include the lipid, membrane and water.

I. Test Concentration -

1. Each microcosm or test chamber will be dosed with 100 µl of each of the 16 PAHs on day 0. The resulting water concentrations will range from 1 µg/L for naphthalene, acenaphthene, acenaphthylene, phenanthrene, fluorene, fluoranthene, pyrene, chrysene, anthracene, benz(a)anthracene, benza(b)fluoranthene and benzo(k)fluoranthene. The remaining PAHs

which include: benz(a)pyrene, benzo(g,h,i)perylene, dibenz(a,h)anthracene and ideno (1,2,3-cd) pyrene, will be less than 1 μg/L because their solubilities range from a low of ≃ 0.0002 μg/L for dibenz(a,h)anthracene. Since this is not a constant renewal system PAH concentrations in water will decline through time.

J. Test Setup and Schedule

1. Test Chambers -

a. Eighteen 950 mL glass (Ball jars) containers sealed with canning lids, having clean aluminum foil on the inside of the lids.

b. chambers are 17.0 cm in height by 9.0 cm i.d.

c. All chambers will be placed in a water bath about 15 cm deep at room temperature

2. SPMDs -

a. See "H 1"

b. SPMDs will be heat sealed (both ends) to obtain a molecular tight enclosure of triolein.

3. Sample Time and Sample Definition

a. Three replicate SPMDs will be sampled at days 1, 4, 7, 14, 28 and 56

b. Water (total volume remaining) will also be sampled in conjunction with each SPMD.

c. SPMD lipid and membrane will be analyzed separately.

d. Total samples from this exposure will be 18 SPMD membranes, 18 SPMD triolein phases and 18 water samples for a grand total of 56 samples.

4. Quality Control

   a. One SPMD blank (freshly prepared SPMD of same size) will be included with replicates from each time period.

   b. One water blank will also be included but not analyzed unless background is problematic.

5. Total SPMDs required for study

   a. 18 for exposure

   b. 6 for blanks

   c. Grand total of 24

K. Test measurements -

A. Sampling Method

1. SPMDs

   a. SPMDs will be collected on Study Days 1, 4, 7, 14, 28, and 56.

   b. Collected SPMDs will be analyzed immediately or placed in solvent rinsed Aluminum foil and frozen (-25°C) until analysis.

2. Test Chamber Water -

   a. Test chamber water will be poured into a 1 L separatory funnel with 100 mL of methylene chloride and extracted immediately.

   b. Water samples will be kept in refrigerator (5°C) until analysis.

B. Method of Chemical Measurement -

1. Residue analysis procedures for PAHs in all samples, which includes stock solutions, SPMDs, and test chamber water.

a. Test Chamber Water Samples -

Using current version of SOP C5.185.

b. SPMD Membrane Samples -

Using current version of SOP C5.185, and SOP C5.173.

c. All Other Samples -

Using current version of SOP C5.185.

APPENDIX B:

STANDARD OPERATING PROCEDURES

For users other than NFCRC staff, this document is for reference only. THIS IS NOT A CITABLE DOCUMENT.

Date Prepared: 11/05/87

Date Revised:

THE CLEANING OF LABWARE FOR USE IN ORGANIC CONTAMINANT RESIDUE ANALYSIS

I. The following procedure should be used to clean glassware.

A. Wash with detergent

B. Rinse with tap water.

C. Rinse with deionized water.

D. Place in annealing oven at 450°C for 10-12 hours.

E. Just prior to use, rinse with methylene chloride and allow to air dry in a fume hood.

II. Teflon items, such as stopcocks, should be rinsed with petroleum ether and allowed to air dry in a fume hood.

Prepared by:

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Approved by:

Chief Chemist

James W. Hogan
Quality Assurance Officer

Date Prepared: 07/16/90

Date Revised: 09/28/90

GAS CHROMATOGRAPHIC PROCEDURE FOR SCREENING AND SEMI-QUANTITATIVE ANALYSIS OF SAMPLE SOLUTIONS PRIOR TO DETERMINATIVE ANALYSIS.

General:

The NFCRC-Columbia analyzes a wide variety of environmental samples for target compounds whose concentrations may vary over several orders of magnitude. Because sample is often limited and the gas chromatographic determinations are sometimes time consuming, a representative number of the sample extracts must be prescreened by sample-conservative, quick GC analysis to estimate analytically appropriate dilution factors. These quick analyses often sacrifice high resolution for rapid (15-60 min/sample) semi-quantitative (or order-of-magnitude) estimates of target compound concentrations. Analytically appropriate sample dilution factors place the target compound concentrations within the range of calibration.

Procedure:

1. Obtain a complete sample inventory and group samples according to location, collection date, etc. Use study plans, protocols, field collection data and experience to assist in the grouping.

2. Adjust all the sample solutions to the same volume.

   a) For most GC/ECD analyses, this volume should contain 1-3 g eq fresh tissue/mL.

   b) For most GC/FID or GC/PID analyses, this volume should contain 10 to 30 g eq of fresh tissue/mL.

   c) Other sample fresh weight/solution volume ratios may be selected based on known or suspected target compound or background component concentrations.

3. Typically, only one sample from each site-group will be screened. When sample collection periods cover several years, then no more than three collection-period samples need be screened; these three samples screened will represent the earliest, middle, and most recent collections.

4. Typically, replicate samples and QA/QC samples will not be screened unless indicated by other requirements specific to the sample set.

5. Calibration of the GC system for screening will require one standard to be analyzed for each 6 to 10 samples and at least one standard/day of screening. This standard will have a target compound concentration within the typical linear instrument calibration range.

6. When sample amount is not limiting, then samples will be screened using autosampling techniques. When sample amounts are very limited, then manual injections during screening analyses may be required.

7. All data will be collected, stored, and integrated using the NFCR-Columbia PE Nelson chromatograph software and hardware, so that sample solutions will not need to be reanalyzed. If data is only collected using a strip chart recorder, then samples with greater than full scale deflection will need to be reanalyzed at a greater GC attenuation.

8. Estimation of target compound(s) concentration(s) in sample solutions will be done by:

   1) visual comparison with the response of the nearest calibration standard, or

   2) using PE Nelson software to rapidly estimate the concentration(s) of the target compound(s) in all screened samples.

Documentation:

The sample name, g eq/mL, injection volume and PE Nelson file name will be entered into the "samples analyzed" section of the pertinent GC instrument log. In addition, the GC system description including the column type, serial#, instrument temperatures and flows, gas types, etc., will be documented in this log book. Although not required, a copy of the PE Nelson files may be archived together with the determinative analysis data files on a project-archive tape or other suitable media. Hard copies of associate data including chromatograms, reports, log book entries, etc. will be kept on file until the results are published or reported. Afterward, the hard copy data will be boxed up and transferred to the Federal Archives and Records Center in Kansas City, KS according to NFCRC SOP B5.147.

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Approved by:

Ted R. Schwartz
Chief Chemist

16-9-90

James W. Hogan
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SOPs Cited:

B5.147 Permanent Archiving of Handwritten Material.

NFCRC SOP C5.173

DATE PREPARED 1/6/92

REVISED DATE 9/14/93

Statement: For users other than NFCRC staff, this document is for reference only. It is not a citable document.

SEMIPERMEABLE MEMBRANE DEVICE (SPMD) CLEANUP AND PREPARATION FOR ANALYSIS

I. **Introduction** - The purpose of this S.O.P. is to delineate key procedural limits of the method yet provide certain specific parameters that currently appear optimal.

II. **Preparation of glassware** - In order to reduce the possibility of the introduction of laboratory contaminants into sample extracts during sample processing or handling, the following procedure is used to clean all glassware:

1. Wash all glassware with laboratory detergent and tap (well water) water. Rinse thoroughly with tap water ($\geq$ 3 times).

2. Rinse each piece of glassware three times with 1 N HCl.

3. Rinse thoroughly with deionized water ($\geq$ 3 times).

4. Rinse three (3) times with acetone (note: all solvents used in the following procedures must be pesticide or nanograde quality, ie. free of contaminants).

5. Rinse three (3) times with hexane.

6. Air dry.

7. Bake in an annealing oven at 475 °C for eight hours.

8. As soon as the glassware is removed from the oven, cover all openings of the glassware with solvent-rinsed (acetone/hexane) aluminum foil and store in drawers or other dust-free areas.

9. Immediately prior to use, rinse each piece of glassware with cyclopentane three (3) times.

Note: Glassware that cannot be annealed, should be rinsed with cyclopentane six (6) times immediately prior to use.

III. **Preparation of SPMDs for dialysis** - When SPMDs are received from field or laboratory exposures, they may have bacteria, algae, sediment, inorganic salts, and other material coating their exterior surface. To prevent this material from

contaminating the dialysate and reducing dialytic recovery, a removal or cleanup step is usually required before dialysis. (Note: Do not touch the SPMD membranes with your hands prior to the following hexane rinse procedure [step 1]). If holes in the SPMDs are visible before cleaning, place dialysis clips on either side of the damaged area and go to step 2. Perform step 1 after heat sealing the damaged SPMD segment.

1. Open the sealed metal or glass container with field deployed SPMDs and pour in ≃ 100 mL hexane. Immediately cover and shake 5 to 10 seconds and then discard the hexane. Care must be used to ensure solvent contact with all SPMD surface. (If the SPMDs are freshly prepared (eg. controls) place in an amber glass jar, add the hexane, shake as above and discard the hexane).

2. Then the SPMDs are placed in a large flat stainless steel pan. Use running tap water (our water is from a deep well and is free of contaminants) and a clean brush and/or hands (ensure gloves or hands are free of soap, powder or oils) to remove all remaining surface material. If the SPMDs have additional seals or loops outside the lipid containment (end) seals, cut them off and discard (this portion of the sampler is usually covered with debris that cannot be easily removed).

3. Drain the water off the SPMDs and swish them in a glass tank containing 1 N HCl for a few seconds (≤ 30 seconds).

4. Thoroughly rinse the SPMDs with tap water to remove the acid.

5. Remove all surface water from the SPMDs, using rinses of acetone followed by isopropanol. Let the rinse solvent evaporate from the SPMDs before dialyzing, by laying the SPMDs on a piece of solvent-rinsed aluminum foil for a few minutes.

Note: Closely inspect the SPMDs for holes as they are cleaned. Water inside the bag is a sure sign of a hole. If there are adequate numbers of undamaged SPMDs, the bad ones can be disposed of. If all are necessary for a sample, make 2 heat seals on either side of the hole. Use the SPMDs as usual but note which ones were damaged.

IV. Dialysis - Canning jars, 1 and 2 quart size, with solvent-rinsed aluminum foil under the screw type lids to create a tight seal are used for the dialysis step. The amount of hexane used for dialysis depends on the total mass of membrane and lipid. A 68" SPMD filled with ≃ 2 mL lipid, requires 250 mL hexane, thus 2 bags require 500 mL, etc. Dialyze the SPMDs at a constant 18 °C for 48 hours. After 48 hours, the membranes are removed from the jar and usually discarded following proper disposal procedures. The dialysate is

quantitatively transferred (using three 3 to 5 mL rinses of hexane) into a round bottom flask equipped with a rotary evaporator trap and reduced to a low volume (3 - 5 mL) on a rotoevaporation system. Then the dialysate is quantitatively transferred to a test tube using several 1 - 2 mL hexane rinses. The dialysate is further reduced to ≃ 1 mL, using high purity nitrogen.

V. **Filtration** - A filtration step is necessary to remove particulates from the extracts before further processing.

1. Poke a little piece of glass fiber filter in a disposable pipet. Pre rinse this "column" with a mixture of 80/20 (v/v) hexane/methylene chloride.

2. Filter the ≃ 1 mL sample through the prepared column.

3. Rinse the tube using several 1 to 2 mL washes of 80/20 and filtering these washes through the column.

4. Rinse the filter with an additional 1 mL of 80/20.

5. Evaporate to 5 mL.

6. Rinse the filter thoroughly between samples.

VI. **Lipid determination** - A lipid analysis can be done on selected dialysates to determine the amount of lipid and/or polyethylene waxes in the samples. Choose at least one sample from each field site plus a control for a lipid analysis. Bring the selected samples to exactly 5 mL. Remove one tenth of the sample (500 μL) and place in a preweighed shell vial. After all the solvent has evaporated, reweigh the vial. By multiplying by 10, the total waxes and lipid per sample can be calculated and the proper GPC method to be used can be determined. If the amount of lipid determined in this step is > 10% of the original lipid mass in the SPMD, a small hole (s) in the membrane is indicated. (Note which samples have had a tenth removed)

VII. **Gel Permeation Chromatography (GPC)** - GPC has proven to be a necessary cleanup step for all SPMD dialysates regardless of which classes of contaminants are targeted for analysis. This is because it is the only innocuous cleanup technique that can remove co-dialyzed lipids and polyethylene waxes.

1. Instrumentation: The GPC instrument consists of the following modular components. 1) Perkin-Elmer Series 410 Solvent Delivery System (pump); 2) Perkin-Elmer ISS-200 Sample Handling System (autoinjector); and 3) Isco Foxy 200 fraction collector. For purposes of calibrating the GPC separation, a UV absorbance detector (254 nm) and strip chart recorder are also used.

2. Columns: There are three options as to which column or columns will be used; the only differences between the columns is their size. The choice will be made by the chemist operating the GPC, and his decision will be based upon the results of the percent lipid analysis. 1) 22.5 mm id X 250 mm column; 2) 7.8 mm id X 600 mm column; or 3) two 7.8 mm id X 600 mm columns in tandem. All of the columns described above contain 10 μm particle size, 10 nm pore size Phenogel. All columns are used in series with the prescribed Phenomenex precolumn.

3. Mobile Phase: 80/20; hexane-dichloromethane (V+V)) pumped at 4.0 mL/min for the wider bore column, or 3.0 mL/ min for the narrower bore columns.

4. GPC Calibration: The chromatographic separation is calibrated by injection of 100 μL of a solution containing DEHP, benzene, biphenyl, sulfur, and pyrene. These materials elute in the sequence in which they were listed. The chromatogram is examined and compared to previously generated calibration chromatograms. If all peaks and separations between peaks appear normal, the retention time of the benzene is measured and recorded. The benzene peak is the basis upon which the retention time that is the end of the dump fraction and the beginning of the collect fraction is chosen. The cutoff point is chosen in such a way that it is as late as possible with the entire benzene peak still being in the collect fraction. The absolute retention times and volumes for the benzene will vary greatly depending upon which GPC column is chosen. Regardless of the column, the end of the dump fraction should be chosen as described. The end of the collected fraction should be chosen in such a way that the total collected fraction is 3.33-times as long as the dump fraction.

5. Sample Preparation: The samples (as prepared in steps V and VI) will be reduced in volume to less than 1 mL under gentle streams of nitrogen. Then CH_2Cl_2 will be used to transfer the samples to conical, 1-mL autoinjector vials. After an entire sample has been quantitatively transferred, a nonane keeper is added to the vial, and most of the CH_2Cl_2 is evaporated away. Then the solution volumes are adjusted to 0.95 mL by the addition of nonane. (So as to ensure that the entire sample is injected, we will program the autoinjector to inject 1.00 mL. It will inject the sample plus 0.05 mL of air.)

6. In accordance with the Perkin-Elmer HPLC and autoinjector operators manuals and the Isco Foxy fraction collector manual, program the modular GPC apparatus to perform the separation in accordance with the results of the calibration chromatogram. Collect the desired fractions on the fraction collector platform in 125 or 250-mL

radiolabeled compound is spiked as described above. The radiolabeled compound is dispersed throughout the SPMD length by smoothing or rubbing the tubing with the palm of the hand. The excess air is removed and the end is resealed with three (3) heat seals. The spiked sample is dialyzed and carried through all steps (counting aliquots) as described above.

4. Field or Trip blank - These are SPMDs that were sent to the field but never removed from their shipping container. The containers were opened on site, resealed and sent back along with exposed samples. Clean these samples as descibed in Part III and carry them through the cleanup procedure as a field sample.

5. The results of all quality control samples are reviewed prior to further sample processing.

IV. Sample fractionation - Following the completion of the procedure(s) presented above, the sample may be processed further for target analytes using the appropriate class specific enrichment procedures.

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9-14-93

Date Prepared: 6/25/92

Date Revised:

For users other than NFCRC staff, this document is for reference only. This is not a citable document.

PROCEDURE FOR THE EXTRACTION, CLEANUP, AND ANALYSES OF POLYCYCLIC AROMATIC HYDROCARBONS (ESPECIALLY PRIORITY POLLUTANT PAHs) IN SEDIMENT AND SOIL SAMPLES

I. SUMMARY

Sediments (or soils) will be air-dried, homogenized, and blended with three times the sediment dry weights of anhydrous Na_2SO_4. The sediment-desiccant mixtures will be packed into 2-cm id extraction columns over 2-cm segments of potassium silicate (KS) and 3-cm segments of Florisil, and extracted with 250 mL of dichloromethane. The sediment extracts will undergo sulfur-removal, then rotary evaporation with a hexane keeper. The solvent compositions will be adjusted to 3 % CH_2Cl_2; 97 % hexane. The solutions will be applied to tandem cartridges, the top cartridges containing KS (henceforth abbreviated the KS ctgs) and the bottom cartridges (henceforth abbreviated the Ag ctgs) containing $AgNO_3$-treated benzenesulfonic acid-bonded phase sorbent. PAHs (especially Priority Pollutant PAHs, but also other PAHs that contain two or more aromatic rings) will be trapped on the Ag ctgs, from which they will be recovered by elution with a benzene-containing solvent. Eluates from the Ag ctgs will be subjected to an additional sulfur-removal treatment. Analyses will be by capillary gas chromatography with photoionization detection (9.5 eV lamp) or possibly by GC/MS.

II. PROTECTION OF ANALYTES.

Many PAH are vulnerable to photodegradation. For this reason, all work involving the samples, their extracts, and PAH standard materials shall be performed under gold lights, or when possible, using amber glassware. When no amber glassware is available, clear glass containers will be used and will be shielded from light with aluminum foil wrappings. Many of the PPPAHs are also somewhat volatile. All evaporative steps will be performed very gently, and at room temperature or cooler.

III. AIR-DRYING OF SEDIMENTS

The sediments (and their accompanying water, if any) will be spread out in Pyrex casserole dishes, which will be loosely covered with aluminum foil, and kept in the fume hood during the air-drying process. One dish containing anhydrous Na_2SO_4 will also be mock air-dried in the hood, and this material will constitute the matrix for the procedural blank(s) which will be extracted, cleaned up, and analyzed in parallel with the sediments. The casserole dishes and all other glassware prescribed in this procedure will have been prepared beforehand as per NFCRC SOP C5.72, then rinsed twice with CH_2Cl_2 immediately before use. The sediments will be dried to equilibrium as in NFCRC SOP C5.126. After having been air-dried, the sediments will be homogenized with a blender and blade apparatus.

Optional

For some sample sets, percent moisture determinations will be performed with small aliquants of the powdered, homogenized sediments. This will entail weighing small portions of the dried, homogenized sediments into tared shell vials, heating the shell vials to 110 °C, reweighing the vials afterward, and calculating the % H_2O by weight differences.

IV. BLENDING OF SEDIMENTS WITH SODIUM SULFATE

Twenty-gram (20.0-g) portions of each dried, homogenized sediment will be weighed into half-pint canning jars, and 60-g (three times the sample weights) of anhydrous sodium sulfate (prepared as per NFCRC SOP C5.8) will be added to them.

> **Note:** The sample size used may vary from project to project. Your supervisor may instruct you to instead extract 5-g, 10-g, or 15-g portion of the sediment. Adjust the amounts of Na_2SO_4 accordingly.

Blender blades will be used to grind the sediments and sodium sulfate together, then the sediments will be allowed to interact with the desiccant for at least two hours before undergoing extraction.

V. EXTRACTION OF SEDIMENT

A. Assembly of columns

Prepare 2-cm i.d. extraction columns as follows. Insert stopcocks in the columns, and over the stopcocks place plugs of glass wool. Use CH_2Cl_2 to thoroughly wash the insides of the columns, the glass wool and the insides of the stopcocks. Rotate the stopcocks several revolutions while the solvent is draining through them.

B. Adding adsorbents to columns

Over the glass wool, deposit 1-cm segments of Na_2SO_4 to form level surfaces upon which to deposit the following adsorbent segments. Add a 3-cm segment of Florisil (prepared as in NFCRC SOP C5.66), and tap the column to settle the Florisil compactly. Over the Florisil deposit KS (prepared as per NFCRC SOP C5.69) to a segment height of 2 cm. Once again, tap the column to settle the adsorbent. Deposit 1-cm segments of Na_2SO_4 over the KS.

C. Packing and spiking of sediments

Dry-pack the sediment-desiccant mixtures into the columns. Spike the procedural internal standards (see below; other spikes, when applicable, should also be applied at this point) onto the sediment at the tops of the columns. Wash the spikes into the matrices with about 3 mL of dichloromethane, then allow 30 min or so for the spiked material to interact with the sediment.

D. Extraction

Deposit layers of Na_2SO_4 over the tops of the spiked sediments, place 300-mL boiling flasks beneath the stopcocks, and gently add 250 mL of CH_2Cl_2 to the reservoirs. Open the stopcocks, and adjust the drip rates to less than 2 mL/min.

VI. SULFUR REMOVAL AND CONCENTRATION OF EXTRACTS

Rotary evaporate the sediment extracts to volumes of about 100 mL, and perform sulfur-removal in accordance with NFCRC SOP C5.70. (This is the first of two copper treatments that the sediment extracts will be subjected to.) Leave the copper balls in the extracts overnight, if possible.

VII. EVAPORATION OF SEDIMENT EXTRACTS

Rotary evaporate the extracts to volumes of about 10 mL, add 3 mL of isooctane, and rotary evaporate to less than 3 mL. Make volume comparisons to identical 300-mL flasks containing 3 mL of isooctane. Transfer the concentrated extracts, through glass fiber filters, into calibrated culture tubes. Rinse the 300-mL flasks with three 3-mL portions of hexane. Filter the sequential rinses into the calibrated culture tubes. Use streams of dry N_2 to evaporate the solutions to about 9.7 mL, and add 0.30 mL of CH_2Cl_2 to each. Vortex thoroughly.

VIII. PREPARATION OF CHROMATOGRAPHY MODULES

A. KS ctgs

Use Baxter-B&J brand, empty, glass solid phase extraction columns, and disks of Whatman brand GF/D glass fiber filters cut out with a no. 8 corkborer. Tamp the disks down flat in the bottoms of the glass columns. Next, deposit KS (fresh from the 130 °C oven) to segment heights of 2 cm (settled levels). Over the KS, deposit anhydrous Na_2SO_4 to segment heights of 0.5 cm. These columns can be prepared in advance, and kept in the 130 °C oven until used. Just before use, wash the KS adsorbent with 5 mL of isooctane.

B. Ag ctgs

1) Prepare a solution of silver nitrate as follows. Into a 100-mL volumetric flask, weigh 8.00 g of $AgNO_3$. Add 9 mL of deionized water, and swirl the flask until the silver nitrate is dissolved. Then dilute to 100 mL with UV grade CH_3CN, and thoroughly shake the flask. Keep the solution refrigerated when not in use. Protect the solution from light at all times.

2) Prepare the Ag ctgs by treating solid phase extraction columns with the silver nitrate solution described above, as follows. Orient cation exchange cartridges (Varian-Analytichem SCX cartridges; 500 mg, 10 mL, cat. no. LR17304) in vertical positions, and add to the reservoir of

each 1.5 mL of the $AgNO_3$ solution. After the last of the solution has descended below the top frit, wash each ctg with 1 mL, then 9 more mL of UV CH_3CN. After the last of the CH_3CN has descended below the top frit, wash each ctg with 1 mL, then 9 more mL of acetone. Once again, after the last of the acetone has descended below the top frit, wash each ctg with 1 mL, then 9 more mL of CH_2Cl_2. Finally, after the last of the CH_2Cl_2 has descended below the top frit, wash each ctg with 5 mL of isooctane. **Important: In the preparation of the Ag ctgs, and in their later use, use gravity-flow only. Do not pressurize or otherwise accelerate the flow rates. The Ag ctgs can be prepared up to a day ahead of time, but should be kept in the freezer in a tightly sealed glass jar if prepared more than two hours ahead of time. The glass jar should also contain a little isooctane to ensure that the ctgs remain solvent-saturated in the interim.**

C. Assembly of ctgs in tandem

Insert the completed Ag ctgs into the mouths of 50-mL centrifuge tubes. Also insert the ends of paper clips into the mouths of the tubes to prevent airtight seals between the outsides of the Ag ctgs and the mouths of the tubes. Next, insert the KS ctgs into the mouths of the Ag ctgs. Insert pieces of wire between the outsides of the KS ctgs and the insides of the Ag ctgs to prevent the formation of airtight seals there, and also to prevent the KS ctgs from entering too far into the mouths of the Ag ctgs. The Ag ctgs will be the modules of the 2-module apparatus that will be the limiting factor in the overall flow rates, so very little of their 10-mL reservoir can afford to be occupied by the KS ctgs.

IX. FRACTIONATION AND CLEANUP ON TANDEM APPARATUS

A. Application of extracts to tandem ctgs

Label the outsides of the Ag ctgs with the corresponding sample numbers. Apply about one-half of each 10-mL extract to the corresponding KS ctg, and allow the liquid to run through into the reservoir of the Ag ctg. The liquid will more slowly pass through the bed of the Ag ctg. When the reservoirs of the Ag ctgs are empty, apply the second halves of the extracts to the KS ctgs. When this liquid has run through both the upper and lower modules, wash the culture tubes that had contained the extract solutions with 1 mL of 3/97; CH_2Cl_2/hexane; V + V, and apply the wash volumes to the KS ctgs.

B. Intermediate elution of tandem ctgs

When all solvent (from A, above) has run through both modules, wash the KS ctgs with three 2-mL portions of 7/93; CH_2Cl_2/hexane; V + V, allowing each discrete washing to descend to the level of the top of the Na_2SO_4 before applying the next. When the reservoirs of the KS ctgs are empty of solvent, remove the KS ctgs. (The KS, glass fiber disk, and Na_2SO_4 should be discarded, and the empty glass columns can be cleaned and reused.) When the reservoirs of the Ag ctgs are empty of solvent, add 2 mL of 7/93; CH_2Cl_2/hexane; V + V, and allow this liquid to pass through the Ag ctgs into the waste reservoirs (50-mL centrifuge tubes).

C. Recovery of PAHs from Ag ctgs

Substitute clean, empty, 10-mL culture tubes for the 50-mL centrifuge tubes beneath the Ag ctgs. Discard the liquid in the 50-mL tubes. Recover the PAHs by washing the Ag ctgs with 10 mL of 10/90; benzene/CH_2Cl_2; V + V. Perform a final copper treatment. Place a small (about 0.5-cm diameter) copper ball in each culture tube. Remove the copper before evaporating the enriched PAH solutions to final volume.

X. PRINCIPLES OF THE CHROMATOGRAPHIC SEPARATION SCHEME

Below are explanations of the principles of the tandem module separation scheme.

A. KS ctgs

The potassium silicate ctg is included to bolster the efficiency of the overall cleanup by removing acidic and polar materials from the extracts. The application solvent, 3/97; CH_2Cl_2/alkane; V + V, is strong enough to wash most of the smaller PAHs directly through the KS ctgs and onto the Ag ctgs. Because this amount and elutropic strength is only marginally adequate to elute some of the larger PAHs from KS, the KS ctgs shall be washed with three 2-mL portions of 7/93; CH_2Cl_2/hexane; V + V, and the resultant effluent liquid from the KS ctgs allowed to run through the Ag ctgs. This will ensure that, before the KS ctgs are discarded, the bigger PAHs will have been eluted from the KS onto the Ag ctgs where the targeted compounds will have become trapped.

B. Ag ctgs

The PAHs exhibit an adsorptive affinity for the $AgNO_3$-treated benzenesulfonic acid sorbent that is proportional to the numbers of aromatic rings in the PAHs. Apparently, the Ag^+ ion, especially when bound to a cation exchange material such as in the prescribed benzenesulfonic cartridges, forms relatively strong complexes with the pi clouds of the conjugated double bonds of PAH molecules. Experience has shown that 10 mL of 12/88; CH_2Cl_2/C_5H_{10}; V + V eluate might just begin to elute naphthalene, the most weakly adsorbed PAH, from an Ag ctg, but that 40 mL of pure CH_2Cl_2 is not enough to completely elute benzo[g,h,i]perylene. This SOP prescribes the use of weaker solvents for extract application and intermediate elution, and thereby incorporates a margin for error. We want to prevent naphthalene from breaking through the Ag ctg until we are ready to collect it along with the larger, more highly retained PAHs. After the KS ctg has been discarded, the Ag ctg will be washed with one 2-mL portion of 7/93; CH_2Cl_2/hexane; V + V, and the resultant effluent will be discarded. This eluant is still inadequate to cause a naphthalene breakthrough, but ensures that additional marginally retained, but non-targeted xenobiotics are discarded before the Ag ctgs are washed with the stronger solvent that will elute the targeted PAHs.

XI. QA/QC SAMPLES, INTERNAL STANDARDS, FINAL VOLUMES, ETC.

A. Procedural blanks

One procedural blank (Na_2SO_4) or a minimum of 5 percent of the total number of sediment samples (whichever is the greater number) shall be processed and analyzed in accompaniment with the sediment samples. Use the sodium sulfate that was mock air-dried in the hood.

B. Control matrix spikes

One control matrix spike or a minimum of 5 percent of the total number of the sediment samples (whichever is the greater number) shall be processed and analyzed in accompaniment with the sediments. NFCRC negative control sediment 354C-1 will serve as the matrix for these. Consult your supervisor as to the identities and quantities of compounds to be spiked.

C. **Unspiked negative control matrix**
One unspiked negative control matrix sample or a minimum of 5 percent of the total number of sediment samples (whichever is the greater number) shall be processed and analyzed in accompaniment with the sediment samples. NFCRC negative control sediment 354C-1 will serve as the matrix for these.

D. **Surrogates and instrumental internal standards**
The spike levels and identities of the surrogates (procedural internal standards) and instrumental internal standards will be chosen on an individual (project-to-project) basis. Consult your supervisor. He or she will also instruct you as to appropriate final volumes for the PAH extracts.

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USE OF THE PHENOGEL COLUMN IN CONJUNCTION WITH THE AUTOINJECTOR-HPLC-FRACTION COLLECTOR TO CLEAN UP DIALYSATES FROM SPMDs AND OTHER TYPES OF EXTRACTS

I. INTRODUCTION

This SOP prescribes, in some detail, how sample cleanups on the Phenogel column will be performed. Included are general instructions in the use of the Perkin-Elmer ISS-200 autosampler, the Perkin-Elmer Series 410 solvent delivery system, and the Isco Foxy 200 fraction collector. This SOP is not intended to supplant the use of the operator's manuals for these instruments, nor is it a substitute for general familiarity with the instruments themselves.

II. INSTRUMENTS, REAGENTS, AND MATERIALS

A. HPLC: Perkin Elmer Series 410.
B. Autosampler: Perkin-Elmer ISS-200.
C. Fraction Collector: Isco Foxy 200.
D. Size Exclusion Columns:
   1. 22.5 mm id X 250 mm;
   2. 7.8 mm id X 600 mm;
   3. two 7.8 mm id X 600 mm columns in tandem;

   NOTE: all columns contain (10 µm particle size; 100 Å pore size) Phenogel. All are used with a 7.8 mm id X 50 mm Phenogel guard column in place between the injector and the column(s).
E. Detector: DuPont Model 842; 254 nm absorbance.
F. Strip Chart Recorder.
G. Mobile Phase: 20:80; CH_2Cl_2/hexane; V+V.
H. Calibration Solution: a nonane solution containing the following: 80 µg/mL DEHP; 600 µg/mL benzene; 5 µg/mL biphenyl: 20 µg/mL sulfur; and 10 µg/mL pyrene.

III. PREPARATION OF SAMPLES FOR A MULTI-SAMPLE GPC CLEANUP

All sample solutions must be filtered through glass fiber filters before they are loaded into the conical vials for autoinjection on the GPC. The samples will normally be received by the GPC operator as approx. 10-mL solutions of hexane in culture tubes. The samples will probably already be filtered when received because the filtrations are most conveniently performed during the transfers from boiling flasks to culture tubes. If not, the filtration procedure is described in another SOP.

Use gentle streams of N_2 to evaporate the solutions in the culture tubes to volumes of about 0.5 mL. Vortex the concentrated solutions, then transfer them to conical vials. Rinse each culture tube with two approx. 0.5-mL portions of hexane. Use the N_2 streams to reduce the solution volumes in the vials, so that the two hexane rinses can be added. After both hexane rinses have been added, continue to blow N_2 into the vials until the solutions are at volumes of about 0.2 mL. Then dilute the samples in the conical vials to volumes of about 0.95 mL with nonane. The vials are ready to be placed in the ISS-200 autoinjector. The autoinjector will load the sample loop with five 200 μL portions of the sample; the last portion will contain about 50 μL of air.

IV. PREPARATION OF GPC FOR A MULTI-SAMPLE RUN

A. On the days that the GPC will be used, the detector and the recorder should be turned on early in the morning to allow them to warm up.

B. The mobile phase should be mixed up next. Always use mobile phase that has been prepared on the day that the GPC is to be run. (Mobile phase left over from previous days will almost certainly contain less than 20 % CH_2Cl_2. The leftover solvent can be economically disposed of by pouring it into the 4-L bottle labelled "SOLVENT FOR RINSE ONLY".) Mix up about 130 % of the volume of mobile phase you calculate you will need for one calibration run plus the number of samples you intend to run. However, mix up a minimum of one liter of mobile phase. After you have separately added the two solvents to the reservoir, swirl the reservoir to mix them well. Gently sparge the mobile phase with helium for exactly 3 min. Then submerge solvent

reservoir **A**'s air stone in the de-gassed mobile phase and screw the cap on tightly.

C. Turn on PE-Series 410 HPLC's power by flipping the toggle switch located on the lower right side. Wait until the instrument has gone through its diagnostics. The liquid crystal display (LCD) will then show you an inactive solvent program. At that point, begin purging the HPLC by hitting the **PURGE** key. Then, hit **F4** to indicate that it is Solvent A that you want to purge with. The LCD will respond by placing a 100 under the percent for Solvent A, and a zero under the percent for Solvent B. Then hit the sequence **F3 2.5 ENTER**. The HPLC will attempt to pump solvent A at 2.5 mL/min. Open the purge valve located in the front-center of the Series 410 HPLC pump. Using a 10-mL Luer Lock syringe, gently draw about 10 mL of solvent A from the purge valve, and dispose of it in the solvent waste. Then close the purge valve. Unless stopped, the HPLC will pump Solvent A indefinitely at 2.5 mL/min in the **PURGE** mode. This flow rate was chosen for purging because it is not excessive, even for the smaller single or tandem 7.8 mm id columns. In order to conserve mobile phase after the HPLC has been purged, hit the sequence **F3 0.4 ENTER**. Mobile phase will be pumped at 0.4 mL/min until the **STOP** or the **F8** (start) key on the Series 410 keyboard is pressed.

D. Pipette 150 μL of the calibration solution into a conical, 1.0-mL GPC autoinjector vial (Chromacol cat. no. 1.1-STVG). Place the vial in position #1 in the ISS-200's autosampler rack. Be sure that there is a conical insert to support this vial in an upright position, and that later, when conical vials containing the actual samples are loaded in the ISS-200's rack, that there is an insert to support each of them.

E. Turn on the ISS-200 autosampler. Press the **Priority Method** button. This will access the Priority Method, which is a special program used just for generating the calibration chromatogram. The purpose in calling up the Priority Method is to Program the ISS-200's method for the calibration autoinjection to the desired specifications. (The Priority Method in the ISS-200's memory will likely already be exactly as needed for this calibration run. However, programming the ISS-200 is done much the same as checking on preexisting programming. If one of the parameters is wrong, the new entry simply overwrites the previous one.) The

proper entries in the Priority Method are as follows. The **FIRST VIAL** is **1**. The **LAST VIAL** is also **1**. The **INJECTION VOLUME** is **100** μL. The **NUMBER OF INJ/VIAL** is **1**. The **ANALYSIS TIME** is **99** minutes (deliberately chosen to be much longer than the total run time of an actual sample). The next three entries do not pertain to GPC or GPC calibration, so the programmer can just hit **ENTER** for each: **TIMED EVENTS**, **STANDARD PROGRAM**, and **DERIVITIZATION PROGRAM**. The ISS-200's screen will then display "ISS-200 Ready", indicating that the Priority Method has been entered.

F. Next, turn your attention back to the Series 410 keyboard. From previous GPC work, we know what the total run time for each sample will be with the column(s) we have chosen. Therefore, we can program the Series 410 at this point. The Series 410 will be where we left it--pumping mobile phase at 0.4 mL/min in the purge mode. Hit the **STOP** button, then **F2** (pump). This will allow you to check the solvent program (or will allow programming of a new method by overwriting the old one). Because a multi-sample run entails continuous pumping of Solvent A in a series of cycles, the program needed is very simple, and looks like this:

SEGMENT	TIME	FLOW	%A	%B	WASH
0	1.0	2.50	100	0	0
1	25.0	2.50	100	0	0

(The example above is for the single 7.8 mm id column. If the tandem 7.8 mm id columns are to be used, the time entry for segment 1 should be <u>40</u> [min]. If the large 22.5 cm id column is used, the time entry for segment 1 should be <u>50</u> [min] and the entry for flow in segments 0 and 1 should be <u>4.0</u> [mL/min].) The **0** segment is not really a segment in the sense that segment **1** is. Upon injection of a sample by the autoinjector, the **1** segment will begin, and Solvent A will be pumped for 25 minutes, then the HPLC will go back to segment **0**. Solvent A will continue to be pumped in segment **0** until the autoinjector tells the HPLC, electronically, that the next sample has been injected, thereby beginning segment **1** again. <u>NOTE</u>: The 25-min (or other) length of segment **1** is deliberately chosen to be much shorter than the actual chromatographic run time of an individual sample. This is done because we don't want the ISS-200 autoinjector to wait for the Series 410's program to finish before beginning to load another sample. Instead, the appropriate total run time will be programmed into and controlled by the

ISS-200. (We will program the ISS-200 autoinjector for the multi-sample run after the calibration chromatogram has been generated.) At this point, it is important to delete all segments beyond segment 1. After this has been done, hit the **STORE** button. The LCD will ask you which method number you want to store the newly created method under. If you choose 1, you may be asked whether you want your new **Method** 1 to overwrite a previously existing Method 1. Signify YES by pushing the **1/Y** key. Then push button **F8**. The Series 410 will then begin the **0** segment of **Method** 1, pumping Solvent A at 2.5 (or 4.0) mL/min, and will be ready for the calibration run.

G. Set the detector attenuation to 4, and the recorder attenuation at 2 mv. Adjust the baseline so that it is 10-20 % of the chartwidth from the right hand margin. Be sure that the pen has adequate ink. Set the chartspeed to 15 cm/h. If the baseline is reasonably stable, begin the calibration by pushing the **START PRIORITY** key on the ISS-200. A few minutes will elapse while 100 μL of calibration solution is loaded into the sample loop. When the actual injection takes place, signify this moment on the chart by hitting the **EVENT MARKER** button on either the detector or on the strip chart recorder. Do not leave the area during the calibration, because you will need to note some retention times. These times can be gotten from the LCD of the Series 410 keyboard. Write on the chromatogram, as it is being generated, the following times:

The apex of the DEHP peak (the first peak)
The apex of the benzene peak (the second peak)
The center of the valley between the benzene peak and the biphenyl peak (the third peak)
The apex of the biphenyl peak
The apex of the sulfur peak (the fourth peak)
The apex of the pyrene peak (the fifth and last peak)

After pyrene has eluted, mobile phase can be conserved by performing the following series of commands. First, stop the mobile phase flow by pushing the **STOP** key on the Series 410's keyboard. Press **PURGE**, and then the sequence **F3 0.4 ENTER**. Mobile phase will be pumped at 0.4 mL/min until the **STOP** key is pressed. Also, after the pyrene has eluted, the detector and the recorder can be turned off, and the chromatogram can be torn off the roll. Write on the chromatogram the following information:

The date;
The mobile phase composition;

The flow rate;
The pressure;
The attenuation;
The chart speed; and--
The column specifications (dimensions and packing)

Check to see whether or not the calibration chromatogram looks remarkably different from previous calibration chromatograms run under identical conditions. The calibration chromatogram is primarily intended to be the sole criterion by which the cutoff point between the dumped fraction and the collected fraction is chosen for the upcoming multi-sample GPC cleanup. However, cursory examination of the calibration chromatograms for anomalies might conceivably prevent valuable dialysates or extracts from being loaded onto a GPC system that is behaving abnormally.

H. Next, program the ISS-200 for the multisample GPC cleanup. In determining how many samples the multi-sample cleanup can encompass, the limiting factor is the number of flasks that can be fit on the collection rack of the Isco Foxy 200 fraction collector. The rack can hold as many as twelve 250-mL boiling flasks, and as many as eighteen 125-mL boiling flasks. Decide which capacity of collection flask is adequate for your multi-sample GPC cleanup. FOR EXAMPLE: When using a single 7.8 mm id column, a total run time per sample of 38 min is more than adequate. The first (approx.) 10 min of this 38 min is devoted to the dump fraction. The remaining (approx.) 28 min (X 2.5 mL/min) constitutes about 70 mL of collected eluate. The 125-mL flasks are appropriate in such a situation; therefore **18** is the number of samples that can be run. (QA/QC requires that one of the 18 be a GPC blank and that one be a GPC spike. Therefore, the multi-sample GPC cleanup can contain 16 "unknown" dialysates, and two additional QA/QC samples.) Press the **METHOD** button on the ISS-200 keyboard. The ISS-200 will ask you what the method number is; reply **1 enter**. The prompt will ask you which autoinjector position the first vial is in. Type in **1 enter**. (Discard the vial presently in autoinjector position no. 1, and that contains calibration solution.) When the ISS-200 prompt next asks you which position the vial containing the last sample is in, type in **17 enter** (or the total: [number of samples plus QA/QC] minus 1). When the ISS-200 prompt asks you the injection volume, type in **1000 enter**. The prompt will then ask how many injections per vial. Answer 1 **enter**. When the ISS-200 asks

for analysis time, type in **31.0 enter** (or the total run time in minutes, minus 7). In response to each of the prompts **TIMED EVENTS**, **STANDARD PROGRAM**, and **DERIVITIZATION PROGRAM**, reply **enter**, (leave them blank) because these functions do not pertain to our GPC cleanups. Then, once again, press the **METHOD** on the ISS-200 keyboard. The ISS-200 will ask you what the method number is; reply **2 enter**. The prompt will ask you what the position of the first sample vial is. Type in **18 enter**. When the ISS-200 prompt next asks you which position the last vial is in, type in **18 enter**. When the ISS-200 prompt asks you the injection volume, type in **1000 enter**. The prompt will then ask how many injections per vial. Answer **1 enter**. When the ISS-200 asks for analysis time, type in **38.0 enter** (or the total run time in minutes). Once again, in response to each of the prompts **TIMED EVENTS**, **STANDARD PROGRAM**, and **DERIVITIZATION PROGRAM**, reply **enter** (leave them blank). The explanation for the use of the two methods is as follows. The total run time in this example has been set at 38 min. The ISS-200 **Method 1**, pertaining to the first 17 samples, has a total run time of 31 min. This means that 31 min after sample 1 has been injected, sample 2 will have begun to be loaded into the sample loop. The ISS-200 loads a 1.0-mL sample in five 200 µL sips, and this loading requires 7 min or more. The Foxy fraction collector will be programmed so that it stops collecting for sample 1 after 38 min. We thereby save mobile phase and time by having samples 2 through 18 loaded while the previous chromatographic runs are still in progress. Next, press the ISS-200's queue key. The queue function arranges the methods that you have entered in an order to be run. For the first method to be run, press **1 enter**, and for the second method to be run, press 2 enter. Leave the ISS-200 as it is, and program the fraction collector.

V. SETTING UP AND PROGRAMMING THE ISCO FOXY 200 FRACTION COLLECTOR

A. Turn on the fraction collector by pushing the power button. Put the required number of boiling flasks on the fraction collector platform. (As previously stated, the platform can accommodate as many as twelve 250- or 300-mL flasks, and as many as eighteen 125-mL flasks.) Arrange the larger flasks in a 2-1-2-1-2-1-2-1 pattern on the platform (looking from front-to-back). The smaller 125-mL flasks should be arranged in a 3-3-3-3-3-3 pattern.

B. Program the Foxy as follows. The first LCD screen will look like this:

Run A **Edit A** **Load** **Quick Review**
Signify that you want to edit program A by pushing Key **B** (the letter located immediately beneath **Edit A**).

The LCD screen will then look like this:
Time/Drop/Vol **Peak** **Delay** **Page 2**
(**The Time/Drop/Vol** and the **Peak** will be flashing.)
Push the **A** key (under **Time/Drop/Volume**) because you will be collecting by time.

The LCD screen will then look like this:
Time **Drop** **Volume** **4 column**
Push the **A** key (underneath the flashing **Time**) to signify that you want to collect by time.

Next, the screen will ask:
Will you use time windows/peak detection in the program?
In response, signify yes (for time windows) by pressing **D** (underneath the flashing **YES**).

The LCD will then show this:
Programming for: **(Press A or C keys)**
Rack 1 (Front Rack) **Rack 2 (Back Rack)**
In response, press **A**, signifying that you will be using the front rack.

Next, the screen will show you this:
Rack 1 (Front Rack) **(Select and Enter)**
Tube Size: Random **Tubes/Rack=X**
If anything other than **random** is shown for tube size, press button A until **Random** is displayed. Then press **enter**. Then the number that had been previously entered for **Tubes/Rack** will begin flashing. Overwrite the number previously entered here and then push **enter** again. The number that you will want here is the number of collection flasks you have.

Then the solvent dropper will move to a position over the

platform that would enable the column effluent to drip into the first collection flask from the previous GPC multi-sample run. The collection flask for the first sample of the upcoming GPC multi-sample run is likely in a different position on the fraction collector platform than the corresponding flask was for the previous run. At this point you will tell the Foxy where each of our collection flasks are located The LED screen shows four arrows pointing in four different directions. The keys **A, B, C, and D** are each located directly underneath one of the arrows. To move the solvent dropper to the left, for example, push button **A** until you have moved the dropper to the left as far as you need to for flask 1. Similarly, use the arrows above **B, C, and D** to move the dropper in any of the other three directions. When you have the dropper placed so that the effluent solvent will fall directly into flask 1, push the **enter** button. Similarly position the dropper for your remaining collection flasks, hitting **enter** after the dropper has been optimally positioned for each. A number in the center of the LED screen will always remind you which collection flask you are positioning the dropper for. When you have positioned the dropper for the last of your collection flasks, hit **enter** one last time.

The LCD screen will then look like this:
Press A for peak collection options, then enter.
Peak=Rack 1 **Nonpeak=Drain**
If these options are shown on the screen, press **enter**. If they are not, press **A** until they are shown, then press **enter**.

The screen will then show you this:
Enter Peak Fraction Size for Rack 1
hr:min:sec 00:29:24
The times illustrated above are only an example of what numbers could have previously been entered here, and will likely need to be overwritten. If, for example, you decided, on the basis of the calibration chromatogram, to dump the first 10.8 min of effluent from every 38-min sample run, your entry should be as follows: **27:12 enter**. The **27** is for min, and the **12** is for sec; if there were three numbers entered instead of two, the number at the left would signify hrs instead of min.

The LCD screen will then look like this:
Program A **Edit Menu** **Page 1 of 3**

Time/Drop/Volume Peak Delay Page 2
Press **B** (directly beneath the flashing **Peak**) signifying that you will be collecting the "peak" in your flasks.

If the screen shows you this,
Press A to select peak detection mode
Mode: Time windows only
Press **enter**. If the screen shows you a peak detection mode other than **Time windows only**, press **A** until it does show time windows only, then press **enter**.

The screen should then show you this:
Window 1 10:48 to 38:00
Exit
The numbers it shows you may differ from this example, however. In our example, we want to dump the first 10.8 min of effluent, and collect the subsequent 27.2 min. The numbers shown above will do that. If the numbers on the screen are appropriate, press **enter** twice. If the numbers on the screen are not the appropriate ones, overwrite them with the correct numbers. Initially, the first number will be flashing, and it will continue flashing until **enter** is pressed, or until it is overwritten and **enter** is pressed. Then the second number will begin flashing, and it is entered or overwritten, then entered in the same fashion.

The next screen will look like this:
Window 2 38.00 to 0.00
Previous Window Exit
Press **C** (underneath **Exit**) because you don't want a second collection window. Then press the **Backstep** key twice in succession.

The screen will show you this:
Press a key to:
Ready A Save As Edit A Quick Review
Press key **B** (directly beneath **Save As**). This is the first step in saving the fraction collecting program you have created.

When the screen shows this:
Press a key to save Program A as:

A full **B full** **C full** **D full**
Press key **A**, to signify that you want to save **Program A** as **A**, and that you want it to overwrite any preexisting **Program A**.

The screen will then look like this:
Pause A **Save As** **Edit A** **Quick Review**
In response, press the **D** key so that all entries can be doublechecked.

The next screen will look like this:
Program A Quick Review
Press enter to review program entries
Press **enter**.

The first quick review screen will then look like this:
Rack 1 Random 18 tubes 27:12
Rack 2 No Rack
The **random** and the **no rack** parameters are critical. The number of tubes (actually flasks) can vary but should be the number you entered earlier. The time given should be the total length of the dump fraction that you entered earlier. If any of the numbers here are inappropriate, you will have to reprogram the Foxy from the start. Hit **enter** if all are appropriate.

The second quick review screen will look like this:
Peak Detection: None **Delay: None**
Windows: One **Nonpeak: Drain**
If the screen looks exactly like this, press **enter**. If there are any discrepancies from this, the Foxy will have to be reprogrammed from the beginning.

The third and final quick review screen will look like this:
Restart: Yes **Initial Waste: No**
Delay: No
If all the parameters are as shown, press the following sequence of keys: **enter**, **backstep**, and **backstep**.

The screen will show you this:

Press a key to:
Ready A **Save As** **Edit A** **Quick Review**
In response, push button **A**, signifying that you want **Program A** ready for the multi-sample GPC run.

Then the screen will look like this:
Ready **Press a key to:**
Run A **Edit A** **Load** **Quick Review**
Press key **A** to run fraction collector **Program A**. The Foxy 200 fraction collector will, at that point, only be waiting for the ISS-200 to electronically signal that the first sample has been injected to begin **Program A**. (The fraction collector clock will begin with the first autoinjection, and will be reset after the fraction collector's total run time has elapsed. The fraction collector timing function will begin again with every subsequent autoinjection.

VI. INITIATING THE MULTI-SAMPLE GPC CLEANUP

You left the Series 410 HPLC pumping mobile phase at 0.4 mL/min in the **PURGE** mode. Stop the flow by pushing the **STOP** button. Then press **F8** (start). This will start the HPLC pumping mobile phase at 2.5 (or 4.0) mL/min in **segment 0** of HPLC **Method 1**. To begin loading the sample in vial 1, press the **START QUEUE** button on the ISS-200 autoinjector.

VII. KEEPING THE GPC LOGBOOK

For each day that samples are run on the GPC, there should be entries made into the logbook. The logbook is associated with the Perkin-Elmer Series 410 HPLC, the Perkin-Elmer ISS-200 Autoinjector, the Isco Foxy 200 Fraction Collector, and with whichever Phenogel columns are used in the size exclusion chromatography.

A. All of the information that was written on the calibration chromatogram should be transcribed into the log. Also, when the day's entries have been made in the logbook, the calibration chromatogram should be taped to the back of the white logbook page.

B. After the above entries have been made, the samples that are run on the GPC should be listed in the logbook. An account

of their injection sequence should also be entered. This is best done by listing the sample numbers next to the numbers of their positions in the ISS-200 autoinjector rack.

C. Any anomalies, observations, etc. should be written in the logbook. This is important because the logbook is occasionally referred to after GC or GC/MS analyses of samples to diagnose analytical problems or abnormalities.

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This is not a citable document.

USE OF MINIATURE POTASSIUM SILICATE COLUMNS TO CLEAN UP DIALYSATES AND CERTAIN OTHER ENVIRONMENTAL SAMPLES

I. INTRODUCTION

The procedure described by this SOP was developed especially for the final cleanup of dialysates that have already been through a size exclusion cleanup. It was designed for samples in which PAHs and PCBs are targeted, however PCDDs/PCDFs and some other halogenated compounds will also be recovered.

II. REAGENTS AND APPARATUS

A. Pasteur pipettes.
B. Glass fiber filters.
C. Sodium sulfate (anhydrous).
D. Potassium silicate ([KS] made according to NFCRC SOP C5.69).
E. Solvent: CH_2Cl_2-hexane; 7 + 93; (V/V)--<u>freshly prepared</u>.

III. PREPARATION OF SAMPLES AND KS COLUMNS

Sample solutions to be cleaned up by this procedure should meet the following criteria. 1) The solutions should be of volumes no greater than 0.5 mL. In practice, solutions of any volume between 0.2 and 0.5 mL can be applied to the little KS columns without the applications being overly broad. For example, the **collect** fractions from size exclusion chromatography (with CH_2Cl_2-hexane; 20 + 80) should be rotary evaporated to volumes of 3 to 5 mL, and transferred to culture tubes with rinses of hexane. The solutions in the culture tubes should then be concentrated, under streams of nitrogen, to volumes of 0.5 mL. 2) The solvent in which the samples are applied should be hexane, isooctane, or if mixed solvents, should be of lesser elutropic strength than is 7/93; CH_2Cl_2/hexane.

The bodies of the KS columns are made from Pasteur pipettes, and the bottom frits are made from glass fiber filters. Cut out a small (about 3 mm x 3 mm) piece of glass fiber filter for each column that you will make. Whatman brand GF/D fiber filters work well for this application. Tamp a piece of the fiber filter down into each Pasteur pipette as far as possible, using another Pasteur pipette as a ramrod. Make marks on the outsides of the pipettes 3.0 cm above the tops of

the pieces of fiber filter, using a metric ruler and a laboratory marker. Orient the empty columns in vertical positions, and add KS (fresh from a 130 °C oven) to the columns until they are filled to the 3-cm marks. Tap the tops of the columns to settle the KS levels; the levels will descend to below the 3-cm marks. Add more KS until the levels again coincide with the 3-cm marks. Then sprinkle anhydrous Na_2SO_4 over the top of the KS to segment heights of 0.25 cm. Fill the reservoirs (those portions of the pipettes above the adsorbent beds) with CH_2Cl_2-hexane; 7 + 93; (V/V), and allow the solvent to flow through the KS via gravity. Fill the reservoirs again, but this time use a Pasteur pipette bulb to force the solvent through the KS at a high flow rate, thereby removing all air from the adsorbent bed. Repeat this step, if necessary. When the above sequence of steps have been performed, and the KS is satisfactorily devoid of air, the solvent levels can be allowed to descend to the levels of the tops of the KS beds. Capillary action will prevent the columns from drying out before you are ready to use them. Dispose of all column effluents thus far obtained.

IV. PERFORMING THE KS CLEANUPS

Place empty graduated culture tubes beneath the pipette columns to receive the analyte-containing effluents. Typically, up to a dozen KS cleanups are performed simultaneously. Below is a description of how a single cleanup is performed. Transfer an extract or dialysate to the reservoir of a pipette column. Wash the culture tube that had contained the extract or dialysate with a 0.5-mL portion of CH_2Cl_2-hexane; 7 + 93; (V/V), and when the liquid level of the initial application has descended to the top of the KS, apply this washing. Repeat this washing-then-application step once more. When the liquid level of the second wash has descended to the top of the KS, wash the KS with enough additional CH_2Cl_2-hexane; 7 + 93; (V/V) that 5.5 mL of eluate are collected in the culture tube beneath the KS. Discard the KS columns after one use.

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Safety Plan for the Fish and Invertebrate Toxicology Section

The U.S. Fish and Wildlife Service Safety Creed (NFCRC Safety Plan, 1986) states that it is the policy of the Fish and Wildlife Service (1) to provide a safe environment for its employees and the public; (2) to seek out and correct unsafe conditions; (3) to preclude unsafe acts through education and on-the-job training; (4) that safety is an integral part of every task; (5) that no job is so important and no service so urgent that sufficient time or precaution cannot be taken to perform them safely (6) that accident prevention is an inherent responsibility or each employee and a measure of each supervisor's efficiency; (7) that safety is essential to the wise use of personnel, material, and equipment. The National Fisheries Contaminant Research Center (NFCRC) is fully committed to that creed.

This SOP is intended to insure the safe conduct of research activities in the Wet Laboratory, the Main Building, the Toxicant Preparation Laboratory (room 35), the Invertebrate Laboratory (room 44), the Fathead Minnow Culture Room, the Fish Culture Annex, the Water Quality Laboratory (room 50), the Pesticide Locker (room 34), and the Walk-in Freezer and Cooler. These locations will be collectively referred to hereafter in this SOP as the "work areas". This SOP may also be useful in other NFCRC laboratories. This SOP is not a comprehensive safty plan for the entire Center. It is meant to ensure the safe conduct of routine activities within the Fish and Invertebrate Toxicology Section. Therefore, all persons using this SOP

should also become thoroughly familiar with the NFCRC Safety Plan (November,1986) as well as more specific SOPs that outline safety guidelines for research activities in the Biology Building East and the High Hazard Assessment Laboratory. This SOP does not cover radiation safety. Safety guidelines pertaining to the storage, handling, and use of radioactive materials are contained in Appendix I of the NFCRC Safety Plan (November, 1986).

I. **General:**

The best clothing and safety devices cannot provide complete protection. All activities must be conducted with concern for personal safety. The following general safety precautions should be followed by all personnel conducting research or research-related activities in the work areas.

1. Gloves, lab coats, safety glasses, and safety shoes shall be worn whenever an employee handles potentially hazardous materials (e.g., contaminants, acids, bases, power tools, or glassware) or performs potentially hazardous activities. The NFCRC will provide this safety equipment.
2. Personnel working in the work areas should know the location of first-aid kits, fire extinguishers, fire blankets, eye-wash stations, safety showers, and chemical spill kits.
3. All accidents resulting in personal injury or property damage must be reported immediately to the Safety Officer or Director.
4. Smoking and consuming food or beverages in the work areas are prohibited.

5. Storage of more than 1 liter of undiluted flammable solvent in the work areas is prohibited. Flammable solvents used in the work areas in excess of 1 liter or contained in 4 liter glass bottles are to be stored in cabinets that are ventilated to the outside. Flammable solvents must be transported in cushioned bottle carriers.
6. Prior to initiating tests with any chemical, information concerning formulation, toxicity, volatility, flammability, and corrosive characteristics of the chemical must be compiled and reviewed by the Lead Biologist and Technician (also see II.A.1 below). This information can be obtained from the Materials Safety Data Sheets which are stored in black binders in room 14.
7. Chemicals are to be disposed of according to procedures outlined in Appendix III of the NFCRC Safety Plan.
8. Adapter plugs should never be used to increase the number of items that can be plugged into any electrical outlet.
9. Radios, tape recorders and other electrical devices not required for studies should not be placed on diluters or above open tanks of water.
10. Radio head phones should not be worn in the work areas.

II. Specific Areas

A. Diluters

Diluters are instruments used to conduct contaminant research. Their proper use requires exercising various safety precautions to avoid personal injuries and property damage(See SOP# B4.7 "Construction, Calibration and Operation of Proportional Diluters" and SOP# F5.1 "Cleanup of Waterbathes and Holding Tanks Upon Completion of Studies

With ^{3}H-Labeled Chemicals"). The following precautions should be used by personnel working with diluters.

1. Before using any work area facility, Lead Biologists shall complete attachment 1 "Study Schedule Planning Form", and have it signed by the appropriate individuals. Lead Biologists shall also post appropriate Study Protocols in a designated area along with any special safety SOP or information concerning the contaminant, species or test conditions.
2. When cleaning debris from diluters with a siphon (water vacuum), the end of the siphon tube should be placed over a trench or effluent drainage manifold (blue pipe in trench). Water (contaminated or clean) should not be siphoned onto the laboratory floor.
3. When fitting glass tubes and latex or other types of tubes together both tubes should be moistened with a water/soap solution so that the tubes will fit together easier. If tubes are extremely difficult to connect, stainless steel fish-cutter's gloves should be worn. These gloves are stored in room 50.
4. All cracked or damaged diluter glass should be cleaned and discarded immediately.
5. After completing a test in a diluter, it should be cleaned and decontaminated (See SOP B4.7 "Construction, Calibration and Operation of Proportional Diluters" and SOP F5.1 "Cleanup of Waterbathes and Holding Tanks Upon Completion of Studies With ^{3}H-Labeled Cemicals") and otherwise left as it was found. This includes disposal of all airstones, tubing, nets, and brushes utilized during the test. In addition, all equipment used during the test such as micromedics,

gangvalves, carboys, etc., should be cleaned and then returned to their respective storage areas.

6. Extremely volatile compounds should not be tested in the work areas.

B. **Drainage Trenches:**

Drainage trenches are used to discharge the large volumes of water generated in the work areas. The following safety precautions are intended to prevent personal injuries and property damage that may result from improper maintenance of drainage trenches and grate covers.

1. All the trench grates are to be maintained firmly in place without the edges extending above the floor surface.
2. Open trenches are to be attended at all times. If an open trench cannot be attended it should be clearly marked "OPEN TRENCH" on all sides.
3. Disposal of solid materials in trenches is prohibited.
4. The trenches or blue drainage manifolds should be thoroughly flushed with well water for at least ten minutes after disposal of flammable solvents or acids.
5. The trenches should be hosed down and debris removed at least twice yearly.

C. **Cleaning-Acid Bath:**

An acid bath is maintained in the Wet Laboratory to facilitate the cleaning of laboratory equipment. The FPRL cleaning-acid contains 38 % hydrochloric acid and detergent. The following safety precautions should be followed when cleaning-acid is used.

1. Hand and eye protection and lab coats should always be worn when using cleaning-acid.
2. Brushes and scrubbing pads should always be removed from the acid bath or other containers of acid, washed thoroughly with well water, and hung to dry.
3. Glassware and other equipment should not be left in the acid bath or other containers of acid for more than one day but preferably not more than a few hours.
4. Buckets and other containers of acid should not be left setting in any laboratory. All used cleaning-acid should be either discarded or poured back into the acid bath. The acid containers should then be thoroughly rinsed and stored in appropriate storage areas.
5. Spilled cleaning acid should be thoroughly washed into the trench with a garden hose.
6. The acid bath should <u>ALWAYS</u> be covered when not in use.

D. **Floor Space:**

The following safety precautions are intended to facilitate safe and efficient use of work area floor space.

1. All hoses are to be placed on a hose rack or rolled up and kept out of walking space when not in use.
2. All tools are to be placed back in their respective storage areas after use.
3. Work area floor space is to be kept free of debris at all times.
4. The work areas are not storage spaces. Storage of items in work areas floor space for long periods of time is prohibited.

5. The work areas floors should be washed down thoroughly or mopped twice monthly. In addition, a general clean-up of the work area should be conducted weekly.

E. **Walk-in Freezer and Cooler:**

The Center maintains a large walk-in freezer and walk-in cooler for storage of samples and other research materials. This equipment may pose severe safety hazards if not used carefully. To avoid accidental injury, the following guidelines must be followed while working inside of the freezer or cooler.

1. While working inside of the walk-in freezer, the door should always be left slightly ajar.
2. If an employee becomes accidently locked inside the freezer, a large ball peen hammer and a crow bar which can be used to escape are stored just inside the freezer door.
3. Hazardous materials are sometimes stored inside the freezer and cooler. These materials should always be handled with caution and care should be taken to avoid contamination of the storage areas.

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PERMANENT ARCHIVING OF HANDWRITTEN MATERIAL

Handwritten material consists of any typed forms or hand recorded data sheets, stored in file folders, that were collected over the period of a study. Data is ready for archiving when a Section decides that a group of files is no longer needed and will probably be accessed no more than once a year.

I. Packing of Files

Packing of files will be in accordance with the Records Management Handbook - Federal Archives and Records Center, #7610-00-298-6904, located in Referring Liasons Office - Linda Sappington.

II. Submitting Request for Transmittal & Receipt

Fill out Standard Form 135 - Records Transmittal & Receipt form and submit to the Federal Archives in Kansas City for them to give you an accession number to be marked on boxes.

III. Final Transfer of Documents

Upon receipt of accession number you will be notified of time allowed for shipment. Include one copy of Records Transmittal and Receipt in the first box and deliver one to Archives Center. The Transferring Liason tries to coordinate multiple Sections transfers for greater efficiency.

IV. Documentation of Materials Sent

Create a file with a listing of handwritten material boxed in each box and submit one copy of list and one copy of Records Transmittal Form to Transferring Liason for later retrieval.

V. Retrieval of Permanently Archived Material

Have the Transferring Liason make a handwritten request or phone call, depending on timeframe, for retrieval of box or file. Retrieval usually takes 24-36 hours to receive.

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PREPARATION OF SEMIPERMEABLE POLYMERIC MEMBRANE DEVICES (SPMDs) WITH MODEL LIPID FOR MONITORING ORGANIC CONTAMINANTS IN WATER

I. Introduction

This is a new approach to environmental contaminant monitoring and certain aspects of the procedure may be refined for years to come. The purpose of this SOP is to delineate key procedural limits of the method and yet provide some specific parameters that appear optimal at this time.

II. Polymeric Membranes

A. Type and configuration - so called nonporous, low-density polyethylene and polypropylene tubings (layflat) and small bore ($\leq$ 2 mm id.) silastic or silicone tubing are suitable for use as semipermeable membrane enclosures (other types of nonporous polymers may be suitable but have not been tested). However, usually polyethylene is used because it is inexpensive and readily available from suppliers. Preferred grades of polyethylene and silicone are those with no additives, blockers, etc., such as untreated (virgin) polyethylene resin and silastic^R (silicone) tubing. The tubing configuration for SPMDs is usually layflat or capillary to permit the construction of SPMDs with high surface area (polymer) to volume (model lipid)

ratios. Recommended polyethylene tubing wall thickness ranges from 0.001" to 0.004" (note: after a wall thickness is chosen, a ± 10% variation in thickness is acceptable) and recommended width of the flattened tubing ranges from 7/8" to 2". In the case of silicone tubing, recommended wall thickness ranges from 0.003" to 0.0095" with a maximum tubing i.d. of 0.058". The length of tubing required for SPMDs is dictated by the amount of model lipid needed to sequester detectable or the desired amounts of contaminants and whether a thin film or turgid (completely filled) configuration of the lipid is desired. For monitoring applications a range of 0.091 to 1.82 g or 0.1 mL to 2 mL of triolein, respectively, are typically used in SPMDs. One mL of triolein (0.91 g) spread into a thin film, requires a 34" length of 1" wide layflat polyethylene tubing or an 18" length of 2" wide layflat tubing. In the case of 0.020" id. silastic tubing, a length of 192" is required for 1 mL of triolein. After a test size configuration is selected, ie. length, width, and polymer, weigh each SPMD tube to the nearest 0.005 g and record data in the appropriate log book or designated data sheet.

B. <u>Removal of potential analytical interferences due to additives , contaminants and small amounts (< 1% w/w) of low molecular weight (polyethylene chains having mw</u>

<u>< 5,000) polyethylene waxes present in the SPMD polymer membrane</u> - The following additives have been identified in low density polyethylene: butylated hydroxytoluene, butylated hydroxy ethyl benzene, Isonox 129, Irganox 1076, erucamide and Irganox 1010. Undoubtedly, low levels of PCBs also exist in all lots of SPMD polymeric tubing. The tubing can be used for SPMDs without removal of possible polymer additives contaminants and other interferring compounds if the user can demonstrate that these materials can be separated from analytes of interest by robust enrichment procedures (caution: polyethylene waxes are stable in the presence of sulfuric acid - silica gel, probably requiring GPC). Otherwise, all SPMD layflat tubing must be batch extracted with nanograde cyclopentane or hexane for 24 to 48 hours to remove most of the aforementioned interferrences. Selection of an optimum extraction time and solvent is based on maximizing the removal of these potentially interfering substances from polyethylene tubes without inducing major changes in flexibility/strength of the membrane and contaminant permeability characteristics.

A typical extraction scheme for layflat polyethylene is as follows: For the cleanup of ca. thirty 70 to 74" by 1" wide layflat tubes, fill a glass thin layer chromatography (TLC) tank with 3-L hexane or

cyclopentane. Put 50 mL of nanograde hexane or cyclopentane inside each tube and slosh the solvent back and forth throughout the length of the tubing for several minutes. Arrange each tube in a loop so the solvent inside contacts the entire length of the tube and then place it in the TLC tank. After all the tubes are in the tank, place a glass-plate weight on top of them to make sure they are submerged. Cover the tank with aluminum foil. Allow batch-solvent extraction to proceed for 24 hours. Afterwards remove the tubes by holding both ends of each tube and then slosh the solvent inside back and forth and finally drain the solvent into a waste solvent container. Place the tubing on solvent rinsed foil to air dry. Turn and fluff occasionally so they will dry as quickly as possible. (Caution: Extended exposure to laboratory air may result in recontaminantion of clean tubes due to the sorption of trace contaminant vapors from the air). Store the cleaned tubes in amber jars with solvent rinsed foil in the caps.

A typical extraction scheme for capillary silastic^{R} tubing is as follows: For the cleanup of ca. twenty 86" by 0.020" i.d. silastic tubes, fill a 1-L glass beaker with 1 L of nanograde hexane or

cyclopentane. Use a teflon squirt bottle to fill each tube with hexane or cyclopentane and then submerge all solvent filled tubes in the extraction solvent using a watch-glass (if needed as a weight). Cover the top of the beaker with solvent rinsed aluminum foil and allow batch-solvent extraction to proceed for 4 hours. Then remove the tubes, draining the solvent inside back into the beaker. Place the tubing on solvent rinsed foil to air dry. Note "caution" statement for polyethylene tubes. Store in the same manner as the polyethylene tubes. Small amounts of interfering substances may still be present in extracted SPMD tubing and should be dealt with by including SPMD reagent blanks in sample sets and open-column GPC cleanup (SOP C, in preparation) prior to analysis.

C. <u>Adequacy of contaminant transport rate through SPMD polymeric enclosures</u> - polymer permeability rates must be evaluated for each lot of polyethylene layflat tubing. This is accomplished by exposing SPMDs containing triolein (95% or 99% pure, 1 mL volume in 34" long, 1" wide, thin film configuration) to 2,2',5,5'-tetrachlorobiphenyl (TCB) in water at 22 ^{o}C. Concentration factors in the triolein of the TCB should reach 3 to 4 X 10^5 at the end of the 1 week exposure. The exposure system used is a glass microcosm (22.5 cm high by 8.3 cm id.) with 1 L of well water. The top is

covered with a slitted (for suspension of SPMD loop) plastic or heavy duty aluminum foil cover.

D. Storage - If the clean tubing is not used immediately after extraction, it should be stored in a contaminant free and an air-tight containers (amber glass recommended) made of UV blocking materials until use.

III. Model Lipid

A. Type and purity - large molecular weight (> 600 daltons) lipids such as triglycerides are usually used in SPMDs. Triolein is the recommended lipid for most pure-lipid SPMDs because it is a liquid down to 4.9 ^{o}C. Currently, 4.9 ^{o}C is the lower limit of the temperature range suitable for the operation of SPMDs with lipid. Triolein or other model lipids should be stored in gas-tight containers, such as glass ampules, at < 5 ^{o}C until used. Clean amber bottles with teflon lined caps are also acceptable containers. Stored under these conditions, a shelf life of at least one year can be expected. The triolein used in SPMDs is usually purchased from Sigma Chemical and is 95 or 99% pure. Another supplier may be used but the purity should be certified at 95 or 99% and the lipid should not contain μg/g concentrations of interfering contaminants.

B. Dialytic removal of background contaminants (< 1μg/g) and small amounts of other lipids from triolein used in

<u>SPMDs</u> - example: Transfer 50 g of 95% triolein at room temperature (22 ^{o}C), using a glass funnel, into a pre-extracted 30" long by 2" wide polyethylene SPMD tube (use care not to contact the first 4 cm of tube with lipid) (II.B.), which has been sealed off on the bottom about 10 cm from the end with a polypropylene dialysis tubing closure (Spectra/Por, Spectrum Medical Indust. Inc.). Continue to hold up the filling end of the tube and fit a dialysis closure on it, about 1 cm from the end, sealing the lipid inside. While continuing to hold up the filling end of the tube, elevate the other end and refit its closure 1 cm from the end, then submerse all but the top portion of the loop (segment between two closures that contains the lipid) into 3 L of nanograde cyclopentane or hexane in a $\simeq$ 4 L glass TLC chamber. Allow the dialysis closures to barely loop over the chamber rim opposite to one another and cover the chamber top with solvent rinsed heavy duty aluminum foil. Note: the foil reduces volatilization of solvent and prevents the tubing ends with closures from falling into the chamber. Allow the lipid containing SPMD to dialyze for 24 hours and then discard the cyclopentane or hexane dialysate into the solvent waste. If necessary (usually cases where model lipid contained high ng/g concentrations of interferring contaminants) add

another 3 L of clean cyclopentane or hexane to the chamber and repeat the process. Afterwards, drain the triolein-solvent mixture inside the SPMD into a clean round bottom glass flask and rotoevaporate off the solvent. Cap the round bottom flask with a glass or teflon stopper and wrap the stopper-flask interface with teflon tape. Alternatively, rinse the clean lipid out of the round bottom flask with three 20 mL aliquots of hexane into a 250 mL amber bottle. Evaporate the solvent off with a clean stream of N_2 and cap bottle with teflon lined cap for storage.

C. Storage - both dialyzed and non-dialyzed triolein must be stored in the dark at < 5 ^{o}C in an airtight glass container. Also see III A.

IV. Preparation of SPMDs for use

A. Method - example 1: One mL of triolein (0.91 g at 25 ^{o}C) is pipetted into one end of an open ended piece of extracted, layflat polyethylene tubing (1" wide by 34" long) positioned horizontally on a laboratory bench top (area is covered with clean aluminum foil). Care is taken not to contact the lipid with the first and last 7.6 cm of tubing. Afterwards, a tubing closure is placed at the 7.0-cm point of the same end to prevent the lipid from migrating back up the tubing. The lipid is squeezed away from the closure and down the length of the tubing to form a continuous, thin film to within

ca. 7.6 cm of the opposite end. For most laboratory exposures, the unclamped end of the tubing is threaded through a slitted piece of 3.2-mm o.d. Teflon tubing (6.2 cm long) so as to form two separate SPMD loops each 32 cm long and then the dialysis closure is disconnected from its original position and used to close both tubing ends ca. 1 cm down from each end. Note: The step used to form more than one loop is not necessary if only a single loop or a straight configuration is desired for the SPMD. For many SPMD applications the heat sealing method is used to enclose lipid in SPMDs. The procedure for heat sealing polyethylene SPMDs is as follows: Plug in the "Seal-a-Meal" or SPMD heat sealer. Temperature setting for the heat sealer should be at least 108 ^{o}C for the bags to be sealed completely. Allow sufficient time ($\geq$ 5 minutes) for the SPMD sealer to reach the preset temperature. Remove the tubing closure from the SPMD. Mark 2" from each end of the bag. Place one end in the "Seal-a-Meal" and at the mark, close the "Seal-a-Meal" for less than a second. Place a second 'safety' seal closer to the open end of the SPMD above the first seal. Repeat this process for the other end of the SPMD. Total length of the tube between the seals should be the length desired for the SPMD. After each end has been sealed, check for leakage by forcing the

lipid up to the seal. Pay special attention to the edges of the seal. Line both ends up and fold them 2 - 3 times. Cut a piece of SPMD tubing (about 1") to go over the fold. Place the piece over the fold and hold (pinch) with forceps near the middle of the SPMD. Place the folded end of the SPMD in the heat sealer. The SPMD has been sealed when the fold "relaxes" or drops to the lower edge of the heat sealer (about 8 seconds). Lightly press the seal together with your fingers. When the seal has cooled, check it by pulling the ends apart. Again, note the edges. Look for any holes in the SPMD due to excessive heating.

Example 2: Take a 1-mL Rainin disposable pipette tip (or equivilant) cut off and discard a 1-cm segment from the end with a scalpel. Take the remaining upper portion and insert into the opening of a similar 200 μL pipet tip creating a 1 mL reservoir with a $\simeq$ 1 mm (0.039") tipped spout. Swell one end ($\simeq$ 1) of an 86" piece of 0.020" i.d. silastic tubing in hexane and insert the prepared 1 mL reservoir pipette tip into the swollen end of the tubing. Suspend the pipette tip reservoir in a vertical position (allowing most of the attached tubing to lie on the foil covered benchtop) using a three-fingered clamp on a ring stand. Add 0.5 mL of triolein to the pipette-tip reservoir and use a 5 mL gas-tight syringe with a 0.03" o.d. blunt tipped

needle (inserted into unattached silastic tupe end) to draw the triolein down the length of the 86" tubing.

Note: The last 1" of each end of the tubing should not contain lipid. Tubing end closure can be accomplished using simple over-hand knots in the tubing or with a Hofman 5/8" metal clamp (Thomas Scientific or other supplier)

B. Storage or transport conditions - place prepared SPMD samplers in a clean, air tight container of glass or steel. If glass is used, an amber color is recommended. Sealed samplers are stored or shipped in a cooler having an inside temperature of < 5 ^{o}C. Below zero is recommended if possible. The maximum time between sampler preparation and environmental use has not been determined but will problably be < 1 month.

C. See NFCR SOP: for recovery of contaminants form field deployed SPMDs.

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APPENDIX C:

QUALITY CONTROL DATA

TABLE I A. PAH MDLs AND MQLs FOR 10 °C FLOW-THROUGH DILUTER STUDY
(Day 4 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	12	12	12	12	0.340	13	15	4.8
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	<2	<2	<2	<2	0.004	2	2	<0.8
fluorene	6	8	6	6	0.732	9	14	2.6
phenanthrene	15	16	16	15	0.741	18	23	<0.8
anthracene	<2	<2	<2	<2	0.004	2	2	<0.8
fluoranthene	202	200	238	213	17.820	267	391	86.0
pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	<2	<2	<2	<2	0.000	2	2	<0.8
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	<2	<2	<2	<2	0.001	2	2	<0.8
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	.2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE I B. PAH MDLs AND MQLs FOR 10 °C FLOW-THROUGH DILUTER STUDY
(Day 7 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	9	9	10	9	0.340	10	13	3.7
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	<2	<2	<2	<2	0.004	2	2	<0.8
fluorene	8	7	8	8	0.535	9	13	3.1
phenanthrene	29	17	23	23	5.024	38	73	9.2
anthracene	4	2	2	3	1.014	6	13	1.1
fluoranthene	151	218	208	192	29.744	281	490	77.5
pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	<2	<2	<2	<2	0.000	2	2	<0.8
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	<2	<2	<2	<2	0.001	2	2	<0.8
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE I C. PAH MDLs AND MQLs FOR 10 °C FLOW-THROUGH DILUTER STUDY
(Day 14 Dilnter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	7	8	8	8	0.455	9	12	3.1
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	<2	<2	<2	<2	0.004	2	2	<0.8
fluorene	12	12	11	12	0.432	13	16	4.7
phenanthrene	49	50	33	44	7.789	67	122	17.7
anthracene	9	7	6	7	1.027	11	18	3.0
fluoranthene	623	609	830	687	100.868	990	1,696	277.1
pyrene	41	41	30	37	4.950	52	87	15.0
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	<2	<2	<2	<2	0.000	2	2	<0.8
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	2	2	2	2	0.041	2	2	0.8
benzo[a]pyrene	2	2	2	2	0.189	3	4	0.9
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	2	12	2	5	4.808	20	53	2.2

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE I D. PAH MDLs AND MQLs FOR 10 °C FLOW-THROUGH DILUTER STUDY
(Day 21 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	3	6	5	5	1.103	8	16	1.8
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	<2	<2	<2	<2	0.004	2	2	<0.8
fluorene	11	11	11	11	0.249	12	13	4.4
phenanthrene	44	31	34	37	5.509	53	92	14.7
anthracene	7	2	5	5	2.148	11	26	1.9
fluoranthene	1,468	1,289	1,318	1,358	78.308	1,593	2,141	547.7
pyrene	4	3	4	4	0.450	5	8	1.5
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	2	5	2	3	1.320	7	16	1.2
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	3	3	3	3	0.082	3	4	1.3
benzo[a]pyrene	2	4	4	3	0.929	6	13	1.3
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	2	12	2	5	4.808	20	53	2.2

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE II A. PAH MDLs AND MQLs FOR 18 °C FLOW-THROUGH DILUTER STUDY
(Day 4 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	8	7	6	7	0.694	9	14	2.9
acenaphthylene	4	2	2	3	0.834	5	11	1.1
acenaphthene	<2 [3]	<2	<2	<2	0.004	2	2	<0.8
fluorene	7	8	8	8	0.294	8	10	3.0
phenanthrene	25	19	17	20	3.615	31	57	8.2
anthracene	4	2	2	3	0.759	5	10	1.1
fluoranthene	218	193	267	226	30.816	318	534	91.1
pyrene	9	10	12	10	1.281	14	23	4.1
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	<2	<2	<2	<2	0.000	2	2	<0.8
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	<2	<2	<2	<2	0.001	2	2	<0.8
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE II B. PAH MDLs AND MQLs FOR 18 °C FLOW-THROUGH DILUTER STUDY
(Day 7 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	---	5	6	5	2.530	13	31	2.2
acenaphthylene	---	<2 [3]	<2	<2	0.002	2	2	<0.8
acenaphthene	---	2	3	2	1.212	6	15	1.0
fluorene	---	10	15	12	6.159	31	74	4.9
pheuanthrene	---	25	19	22	10.670	54	129	8.8
anthracene	---	3	2	3	1.320	7	16	1.0
fluoranthene	---	267	260	263	124.231	636	1,506	106.2
pyrene	---	14	12	13	6.116	31	74	5.2
benz[a]anthracene	---	<2	<2	<2	0.005	2	2	<0.8
chrysene	---	6	2	4	2.273	11	26	1.5
benzo[b]fluoranthene	---	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	---	<2	<2	<2	0.001	2	2	<0.8
benzo[a]pyrene	---	2	2	2	1.050	5	13	0.9
indeno[123cd]pyrene	---	4	2	3	1.512	7	18	1.1
dibenz[ah]anthracene	---	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	---	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE II C. PAH MDLs AND MQLs FOR 18 °C FLOW-THROUGH DILUTER STUDY
(Day 14 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	6	3	6	5	1.461	9	19	2.0
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	3	2	2	2	0.424	4	7	0.9
fluorene	10	10	13	11	1.349	15	24	4.4
phenanthrene	30	41	37	36	4.472	49	81	14.5
anthracene	5	7	6	6	1.103	9	17	2.4
fluoranthene	641	702	791	711	61.483	896	1,326	286.9
pyrene	20	23	13	19	4.055	31	59	7.6
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	5	2	2	3	1.273	7	16	1.2
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	2	2	2	2	0.141	3	4	0.8
benzo[a]pyrene	8	2	2	4	2.876	13	33	1.6
indeno[123cd]pyrene	9	2	2	4	3.441	15	39	1.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE II D. PAH MDLs AND MQLs FOR 18 °C FLOW-THROUGH DILUTER STUDY
(Day 21 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean ng/g
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	
naphthalene	5	5	6	5	0.330	6	9	2.2
acenaphthylene	7	2	2	4	2.121	10	25	1.4
acenaphthene	2	5	4	4	1.307	7	17	1.4
fluorene	11	12	13	12	1.020	15	22	4.8
phenanthrene	2	41	55	33	22.496	100	258	13.2
anthracene	6	6	6	6	0.216	7	8	2.4
fluoranthene	1,103	1,524	1,707	1,445	252.844	2,203	3,973	582.5
pyrene	25	16	77	39	27.079	120	310	15.8
benz[a]anthracene	<2 [3]	<2	<2	<2	0.005	2	2	<0.8
chrysene	<2	<2	<2	<2	0.000	2	2	<0.8
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	2	3	2	2	0.327	3	6	1.0
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	2	19	2	8	8.014	32	88	3.1
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	2	3	2	2	0.419	4	7	1.0

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE III A. PAH MDLs AND MQLs FOR 26 °C FLOW-THROUGH DILUTER STUDY
(Day 4 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	8	10	9	9	0.974	12	19	3.6
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	2	2	2	2	0.047	2	3	0.8
fluorene	7	6	8	7	0.579	9	13	2.8
phenanthrene	24	20	24	23	1.934	29	42	9.2
anthracene	3	3	3	3	0.163	4	5	1.3
fluoranthene	40	115	71	75	30.939	168	384	30.3
pyrene	18	15	16	16	1.342	20	30	6.6
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	<2	<2	<2	<2	0.000	2	2	<0.8
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	<2	<2	<2	<2	0.001	2	2	<0.8
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE III B. PAH MDLs AND MQLs FOR 26 °C FLOW-THROUGH DILUTER STUDY
(Day 7 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
uaphthalene	7	6	9	7	1.190	11	19	2.9
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	2	3	2	2	0.236	3	5	0.9
fluorene	9	6	6	7	1.322	11	20	2.7
phenanthrene	30	30	27	29	1.377	33	43	11.6
anthracene	4	4	4	4	0.205	5	6	1.6
fluoranthene	128	180	83	130	39.919	250	529	52.5
pyrene	27	24	21	24	2.547	32	49	9.7
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	<2	<2	<2	<2	0.000	2	2	<0.8
benzo[b]fluoranthene	<2	<2	<2	<2	0.004	2	2	<0.8
benzo[k]fluoranthene	<2	<2	<2	<2	0.001	2	2	<0.8
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE III C. PAH MDLs AND MQLs FOR 26 °C FLOW-THROUGH DILUTER STUDY
(Day 14 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	6	5	6	6	0.432	7	10	2.3
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	2	2	2	2	0.170	3	4	0.9
fluorene	9	8	9	9	0.368	10	12	3.5
phenanthrene	35	31	35	33	1.948	39	53	13.5
anthraceue	5	5	4	5	0.262	5	7	1.8
fluoranthene	263	79	133	158	77.030	389	928	63.8
pyrene	28	21	25	25	2.780	33	52	9.9
benz[a]anthracene	<2	<2	<2	<2	0.005	2	2	<0.8
chrysene	8	9	13	10	2.330	17	33	4.0
benzo[b]fluoranthene	2	5	2	3	1.179	6	15	1.1
benzo[k]fluoranthene	4	2	2	3	1.037	6	13	1.1
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE III D. PAH MDLs AND MQLs FOR 26 °C FLOW-THROUGH DILUTER STUDY
(Day 21 Diluter Control SPMDs)

PAHs	ng / SPMD [1]							Mean
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]	MDL	MQL	ng/g
naphthalene	2	4	5	3	1.027	6	14	1.3
acenaphthylene	<2 [3]	<2	<2	<2	0.002	2	2	<0.8
acenaphthene	<2	<2	<2	<2	0.004	2	2	<0.8
fluorene	7	9	8	8	0.726	10	15	3.3
phenanthrene	31	39	33	34	3.318	44	67	13.8
anthracene	4	5	5	5	0.356	6	8	2.0
fluoranthene	34	142	84	87	38.436	202	471	34.9
pyrene	23	31	31	28	2.919	37	57	11.4
benz[a]anthracene	3	3	4	3	0.419	4	7	1.3
chrysene	8	7	8	8	0.363	9	11	3.2
benzo[b]fluoranthene	6	9	8	8	1.068	11	18	3.1
benzo[k]fluoranthene	<2	<2	<2	<2	0.001	2	2	<0.8
benzo[a]pyrene	<2	<2	<2	<2	0.005	2	2	<0.8
indeno[123cd]pyrene	<2	<2	<2	<2	0.006	2	2	<0.8
dibenz[ah]anthracene	<2	<2	<2	<2	0.011	2	2	<0.8
benzo[ghi]perylene	<2	<2	<2	<2	0.001	2	2	<0.8

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE IV A. MDLs AND MQLs FOR PAH FRESHWATER STATIC EXPOSURES (Control SPMD Membranes)

PAHs	ng / SPMD [1]										Study Mean ng/g
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev. [2]	Study MDL	Study MQL	
naphthalene	<10 [3]	<10	<10	<10	<10	10	10	0.001	10	10	22
acenaphthylene	<10	<10	<10	<10	<10	<10	<10	0.006	<10	<10	<22
acenaphthene	<10	<10	<10	<10	<10	<10	<10	0.002	<10	<10	<22
fluorene	<10	<10	14	<10	<10	<10	11	0.032	11	11	24
phenanthrene	<10	<10	<10	<10	<10	95	<10	0.006	<10	<10	<22
anthracene	<10	<10	<10	<10	<10	95	<10	0.007	<10	<10	<22
fluoranthene	92	<10	<10	<10	12	40	29	30.021	119	329	64
pyrene	36	<10	<10	<10	61	136	44	45.177	179	495	97
benz[a]anthracene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
chrysene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
benzo[b]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
benzo[k]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
benzo[a]pyrene	35	<10	<10	<10	<10	<10	14	0.010	14	14	32
indeno[123cd]pyrene	57	<10	<10	<10	<10	<10	18	0.012	18	18	40
dibenz[ah]anthracene	<10	<10	<10	<10	<10	<10	<10	0.001	<10	<10	<22
benzo[ghi]perylene	<10	<10	<10	<10	<10	<10	<10	0.015	<10	<10	<22

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).
[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XX).
[3] < = Background or samples with values less than average noise peak area of 300 or 10 ng/0.5 mL sample.

TABLE IV B. MDLs AND MQLs FOR PAH FRESHWATER STATIC EXPOSURES (Control SPMD Lipids)

PAHs	ng / SPMD [1]										Study Mean ng/g
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev. [2]	Study MDL	Study MQL	
naphthalene	<10 [3]	<10	<10	<10	<10	<10	<10	0.001	<10	<10	<110
acenaphthylene	<10	<10	<10	<10	<10	<10	<10	0.006	<10	<10	<110
acenaphthene	<10	<10	<10	<10	<10	<10	<10	0.002	<10	<10	<110
fluorene	<10	16	<10	<10	<10	<10	11	0.032	11	11	120
phenanthrene	<10	<10	<10	<10	<10	<10	<10	0.006	<10	<10	<110
anthracene	<10	<10	<10	<10	<10	<10	<10	0.007	<10	<10	<110
fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.003	<10	<10	<110
pyrene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<110
benz[a]anthracene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<110
chrysene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<110
benzo[b]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<110
benzo[k]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<110
benzo[a]pyrene	<10	<10	<10	<10	<10	<10	<10	0.010	<10	<10	<110
indeno[123cd]pyrene	<10	<10	<10	<10	<10	<10	<10	0.012	<10	<10	<110
dibenz[ah]anthracene	<10	<10	<10	<10	<10	<10	<10	0.001	<10	<10	<110
benzo[ghi]perylene	<10	20	<10	<10	<10	<10	12	0.015	12	12	128

[1] SPMDs = 10 cm x 2.54 cm with 0.1 mL (0.09g) triolein and 0.45g membrane (Total SPMD mass = 0.54g).
[2] Standard Deviation of samples were derived from the Standard Deviation of the lowest Calibration Standard (Table XX).
[3] < = Background or samples with values less than average noise peak area of 300 or 10 ng/0.5 mL sample.

TABLE V A. SPMD SPIKE VERIFICATION SAMPLES FOR 10 °C FLOW-THROUGH DILUTER STUDY

PAHs	ng / Recovered [1]						
	Day 4 Rep 1	Day 7 Rep 2	Day 14 Rep 3	Day 21 Rep 4	Mean	Std Dev.	C.V.
naphthalene	160	160	167	169	164	4	2
acenaphthylene	176	156	167	178	169	9	5
acenaphthene	160	163	160	165	162	2	1
fluorene	150	150	160	152	153	4	3
phenanthrene	186	177	189	193	186	6	3
anthracene	172	158	200	201	183	19	10
fluoranthene	184	184	219	200	197	14	7
pyrene	193	179	183	181	184	5	3
benz[a]anthracene	196	191	200	203	198	4	2
chrysene	241	253	235	269	250	13	5
benzo[b]fluoranthene	187	187	194	199	192	5	3
benzo[k]fluoranthene	228	194	261	235	229	24	10
benzo[a]pyrene	192	203	218	209	206	10	5
indeno[123,cd]pyrene	190	197	214	216	204	11	5
dibenz[ah]anthracene	203	209	212	215	210	4	2
benzo[ghi]perylene	151	169	154	173	162	9	6

[1] Nominal amount spiked was 200 ng per PAH.

TABLE V B. SPMD SPIKE VERIFICATION SAMPLES FOR 18 °C FLOW-THROUGH DILUTER STUDY

PAHs	ng / Recovered [1]						
	Day 4 Rep 5	Day 7 Rep 6	Day 14 Rep 7	Day 21 Rep 8	Mean	Std Dev.	C.V.
naphthalene	164	172	168	168	168	3	2
acenaphthylene	156	151	155	155	154	2	1
acenaphthene	130	164	156	157	152	13	8
fluorene	120	155	145	155	144	14	10
phenanthrene	183	181	187	189	185	3	2
anthracene	157	189	186	202	183	16	9
fluoranthene	197	177	203	204	195	11	6
pyrene	165	185	173	191	178	10	6
benz[a]anthracene	202	203	197	199	200	2	1
chrysene	258	253	219	250	245	15	6
benzo[b]fluoranthene	206	205	200	201	203	3	1
benzo[k]fluoranthene	213	241	213	229	224	12	5
benzo[a]pyrene	86	218	234	252	198	65	33
indeno[123,cd]pyrene	213	207	211	213	211	3	1
dibenz[ah]anthracene	215	198	209	223	211	9	4
benzo[ghi]perylene	175	189	173	163	175	9	5

[1] Nominal amount spiked was 200 ng per PAH.

TABLE V C. SPMD SPIKE VERIFICATION SAMPLES FOR 26 °C FLOW-THROUGH DILUTER STUDY

PAHs	ng / Recovered [1]						
	Day 4 Rep 9	Day 7 Rep 10	Day 14 Rep 11	Day 21 Rep 12	Mean	Std Dev.	C.V.
naphthalene	163	169	171	163	167	3	2
acenaphthylene	153	157	159	168	159	6	3
acenaphthene	158	167	165	164	163	3	2
fluorene	154	158	156	143	153	6	4
phenanthrene	182	184	186	185	184	2	1
anthracene	197	176	169	176	180	10	6
fluoranthene	220	229	228	199	219	12	5
pyrene	157	176	184	177	174	10	6
benz[a]authracene	190	197	208	206	200	7	4
chrysene	257	266	264	268	264	4	2
benzo[b]fluoranthene	197	206	207	207	204	4	2
benzo[k]fluoranthene	220	231	234	261	237	15	6
benzo[a]pyrene	199	221	241	222	221	15	7
indeno[123,cd]pyrene	196	236	222	227	220	15	7
dibenz[ah]anthracene	197	220	229	215	215	12	6
benzo[ghi]perylene	164	147	176	183	167	14	8

[1] Nominal amount spiked was 200 ng per PAH.

TABLE VI. SUMMARY OF SPMD SPIKE VERIFICATION SAMPLES FOR FLOW-THROUGH DILUTER STUDY

PAHs	ng / Recovered [1]					
	10 °C Mean	18 °C Mean	26 °C Mean	Study Mean	Study Std Dev.	Study C.V.
naphthalene	164	168	167	166	2	1
acenaphthylene	169	154	159	161	6	4
acenaphthene	162	152	163	159	5	3
fluorene	153	144	153	150	4	3
phenanthrene	186	185	184	185	1	0
anthracene	183	183	180	182	2	1
fluoranthene	197	195	219	204	11	5
pyrene	184	178	174	179	4	2
benz[a]anthracene	198	200	200	199	1	1
chrysene	250	245	264	253	8	3
benzo[b]fluoranthene	192	203	204	200	6	3
benzo[k]fluoranthene	229	224	237	230	5	2
benzo[a]pyrene	206	198	221	208	10	5
indeno[123,cd]pyrene	204	211	220	212	7	3
dibenz[ah]anthracene	210	211	215	212	2	1
benzo[ghi]perylene	162	175	167	168	5	3
Mean	190	189	195	192	3	1

[1] Nominal amount spiked was 200 ng per PAH.

TABLE VII A. RECOVERY OF PAHs FROM SPIKED CONTROL SPMDs FROM 10 °C FLOW-THROUGH DILUTER STUDY

PAHs [2]	ng / SPMD [1]							Mean ng/g
	Day 4 Rep 1	Day 7 Rep 2	Day 14 Rep 3	Day 21 Rep 4	Mean	Std. Dev.	C.V.	
naphthalene	19	39	42	40	35	9	27	14
acenaphthylene	73	65	79	73	73	5	7	29
acenaphthene	87	73	79	73	78	6	7	31
fluorene	88	81	104	90	91	8	9	37
phenanthrene	100	100	127	129	114	14	12	46
anthracene	100	100	120	119	110	10	9	44
fluoranthene	ND [3]	ND	ND	ND	ND	---	---	ND
pyrene	116	116	110	166	127	22	18	51
benz[a]anthracene	144	117	128	138	132	10	8	53
chrysene	126	149	166	160	151	15	10	61
benzo[b]fluoranthene	112	107	123	136	119	11	9	48
benzo[k]fluoranthene	132	112	141	144	132	12	9	53
benzo[a]pyrene	126	122	134	136	129	6	5	52
indeno[123,cd]pyrene	110	104	116	120	112	6	5	45
dibenz[ah]anthracene	100	105	118	123	111	9	8	45
benzo[ghi]perylene	101	99	109	120	107	8	8	43

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Spike Verification table (V A.) amounts spiked into Control SPMD.

[3] ND = (<MQL of SPMD Controls).

TABLE VII B. RECOVERY OF PAHs FROM SPIKED CONTROL SPMDs FROM 18 °C FLOW-THROUGH DILUTER STUDY

PAHs [2]	ng / SPMD [1]							Mean ng/g
	Day 4 Rep 5	Day 7 Rep 6	Day 14 Rep 7	Day 21 Rep 8	Mean	Std. Dev.	C.V.	
naphthalene	58	55	39	61	53	9	16	22
acenaphthylene	66	101	87	125	95	21	23	38
acenaphthene	72	87	75	89	81	7	9	33
fluorene	109	116	91	127	110	13	12	45
phenanthrene	159	135	99	126	130	22	17	52
anthracene	108	113	83	99	101	11	11	41
fluoranthene	ND [3]	ND	ND	ND	ND	---	---	ND
pyrene	122	114	85	<39	90	33	36	36
benz[a]anthracene	139	157	139	156	147	9	6	59
chrysene	128	138	127	160	138	13	10	56
benzo[b]fluoranthene	125	136	102	131	123	13	11	50
benzo[k]fluoranthene	140	140	111	130	130	12	9	53
benzo[a]pyrene	134	133	122	150	135	10	8	54
indeno[123,cd]pyrene	122	113	76	98	102	18	17	41
dibenz[ah]anthracene	85	117	84	119	101	17	17	41
benzo[ghi]perylene	108	105	95	123	108	10	10	43

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Spike Verification table (V B.) amounts spiked into Control SPMD.

[3] ND = (<MQL of SPMD Controls).

TABLE VII C. RECOVERY OF PAHs FROM SPIKED CONTROL SPMDs FROM 26 °C FLOW-THROUGH DILUTER STUDY

PAHs [2]	ng / SPMD [1]							Mean ng/g
	Day 4 Rep 9	Day 7 Rep 10	Day 14 Rep 11	Day 21 Rep 12	Mean	Std. Dev.	C.V.	
naphthalene	63	87	53	72	69	13	18	28
acenaphthylene	90	91	80	81	85	5	6	34
acenaphthene	104	103	84	92	96	8	9	39
fluorene	84	85	70	64	76	9	12	31
phenanthrene	107	123	84	96	102	15	14	41
anthracene	93	99	83	83	90	7	7	36
fluoranthene	ND [3]	ND	ND	ND	ND	---	---	ND
pyrene	102	98	83	79	90	10	11	36
benz[a]anthracene	118	126	95	104	111	12	11	45
chrysene	144	140	119	124	132	11	8	53
benzo[b]fluoranthene	113	120	97	100	107	10	9	43
benzo[k]fluoranthene	136	148	114	132	132	12	9	53
benzo[a]pyrene	122	132	109	118	120	8	7	48
indeno[123,cd]pyrene	107	117	89	94	102	11	11	41
dibenz[ah]anthracene	116	130	103	117	116	9	8	47
benzo[ghi]perylene	113	121	95	109	110	9	9	44

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Spike Verification table (V C.) amounts spiked into Control SPMD.

[3] ND = (<MQL of SPMD Controls).

TABLE VII D: SPIKE RECOVERIES FOR WATER IN FLOW-THROUGH DILUTER EXPOSURES

PAHs	Spike Verification ng	ng/ Sample [1]							
		10 °C Day 0	10 °C Day 21	10 °C Average	18 °C Day 0	18 °C Day 21	18 °C Average	26 °C Day 0	Study Mean
naphthalene	195	157	138	148	95	101	98	118	121
acenaphthylene	184	155	152	154	111	129	120	122	132
acenaphthene	188	159	167	163	108	118	113	137	137
fluorene	144	176	234	205	109	125	117	141	154
phenanthrene	158	166	166	166	112	128	120	187	158
anthracene	188	166	159	163	114	119	117	137	139
fluoranthene	156	156	162	159	133	158	145	161	155
pyrene	152	151	152	152	152	131	141	143	145
benz[a]anthracene	168	169	184	176	133	155	144	182	168
chrysene	174	172	195	183	110	134	122	151	152
benzo[b]fluoranthene	198	166	176	171	141	173	157	195	174
benzo[k]fluoranthene	200	167	174	170	129	140	134	156	153
benzo[a]pyrene	195	178	178	178	137	183	160	170	169
indeno[123,cd]pyrene	200	171	172	172	112	157	134	176	161
dibenz[ah]anthracene	220	175	176	176	127	137	132	180	163
benzo[ghi]perylene	226	167	188	178	111	126	119	179	158

[1] Sample = Amount in Spike Verification column added to 2L Control water and processed as a water sample.

TABLE VIII A. RECOVERY OF PAHs FROM SPIKED CONTROL SPMDs FROM 10 °C FLOW-THROUGH DILUTER STUDY

PAHs [1]	% Recovery						
	Day 4	Day 7	Day 14	Day 21	Mean	Std Dev.	C.V.
naphthalene	12	24	25	24	21	6	26
acenaphthylene	41	42	48	41	43	3	6
acenaphthene	54	45	50	44	48	4	8
fluorene	59	54	65	59	59	4	6
phenanthrene	54	57	67	67	61	6	9
anthracene	58	63	60	59	60	2	3
fluoranthene	ND [2]	ND	ND	ND	ND	---	---
pyrene	60	65	60	92	69	13	19
benz[a]anthracene	73	61	64	68	67	5	7
chrysene	52	59	71	59	60	7	11
benzo[b]fluoranthene	60	57	63	68	62	4	7
benzo[k]fluoranthene	58	58	54	61	58	2	4
benzo[a]pyrene	65	60	62	65	63	2	4
indeno[123,cd]pyrene	58	53	54	56	55	2	3
dibenz[ah]anthracene	49	50	55	57	53	3	6
benzo[ghi]perylene	67	59	71	69	67	5	7

[1] Spike Verification table (V A.) amounts spiked into Control SPMD.
[2] ND = (<MQL of SPMD Controls).

TABLE VIII B. RECOVERY OF PAHs FROM SPIKED CONTROL SPMDs FROM 18 °C FLOW-THROUGH DILUTER STUDY

PAHs [1]	% Recovery						
	Day 4	Day 7	Day 14	Day 21	Mean	Std Dev.	C.V.
naphthalene	35	32	23	37	32	5	17
acenaphthylene	42	67	56	81	62	14	23
acenaphthene	55	53	48	57	53	3	6
fluorene	91	75	63	82	78	10	13
phenanthrene	87	74	53	67	70	12	18
anthracene	69	60	45	49	56	9	17
fluoranthene	ND [2]	ND	ND	ND	ND	---	---
pyrene	74	62	49	ND	62	10	16
benz[a]anthracene	69	77	70	78	74	4	6
chrysene	50	55	58	64	57	5	9
benzo[b]fluoranthene	61	66	51	65	61	6	10
benzo[k]fluoranthene	65	58	52	57	58	5	8
benzo[a]pyrene	156	61	52	60	82	43	52
indeno[123,cd]pyrene	57	55	36	46	48	8	17
dibenz[ah]anthracene	39	59	40	53	48	8	18
benzo[ghi]perylene	62	55	55	76	62	8	14

[1] Spike Verification table (V B.) amounts spiked into Control SPMD.
[2] ND = (<MQL of SPMD Controls).

TABLE VIII C. RECOVERY OF PAHs FROM SPIKED CONTROL SPMDs FROM 26 °C FLOW-THROUGH DILUTER STUDY

PAHs [1]	% Recovery						
	Day 4	Day 7	Day 14	Day 21	Mean	Std Dev.	C.V.
naphthalene	39	52	31	44	41	8	18
acenaphthylene	59	58	50	48	54	5	9
acenaphthene	66	62	51	56	59	6	10
fluorene	55	54	45	45	50	5	9
phenanthrene	59	67	45	52	56	8	15
anthracene	47	56	49	47	50	4	7
fluoranthene	ND [2]	ND	ND	ND	ND	---	---
pyrene	65	56	45	45	53	8	16
benz[a]anthracene	62	64	46	51	56	8	14
chrysene	56	52	45	46	50	5	9
benzo[b]fluoranthene	57	58	47	48	53	5	10
benzo[k]fluoranthene	62	64	49	50	56	7	12
benzo[a]pyrene	62	60	45	53	55	6	12
indeno[123,cd]pyrene	55	49	40	41	46	6	13
dibenz[ah]anthracene	59	59	45	55	54	6	10
benzo[ghi]perylene	69	83	54	59	66	11	16

[1] Spike Verification table (V C.) amounts spiked into Control SPMD.
[2] ND = (<MQL of SPMD Controls).

TABLE VIII D: SPIKE RECOVERIES FOR WATER IN FLOW-THROUGH DILUTER EXPOSURES (Study Summary)

PAHs	% Recovery			
	10 °C Average	18 °C Average	26 °C Average	Study Mean
naphthalene	76	50	61	62
acenaphthylene	84	65	66	72
acenaphthene	87	60	73	73
fluorene	142	81	98	107
phenanthrene	105	76	119	100
anthracene	86	62	73	74
fluoranthene	102	93	103	100
pyrene	100	93	94	95
benz[a]anthracene	105	86	108	99
chrysene	105	70	87	88
benzo[b]fluoranthene	86	80	99	88
benzo[k]fluoranthene	85	67	78	77
benzo[a]pyrene	91	82	87	87
indeno[123,cd]pyrene	86	67	88	80
dibenz[ah]anthracene	80	60	82	74
benzo[ghi]perylene	79	53	79	70

TABLE IX. SPMD AND WATER SPIKE VERIFICATION SAMPLES FOR PAH FRESHWATER STATIC EXPOSURES

PAHs	ng / Recovered [1]								
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev.	Study C.V.
naphthalene	1,017.2	855.2	895.0	923.4	926.1	906.7	920.6	49.2	5.3
acenaphthylene	898.2	847.5	858.3	833.5	880.7	871.5	871.2	19.7	2.3
acenaphthene	968.2	863.8	896.3	843.3	911.6	967.2	910.0	37.8	4.2
fluorene	870.6	937.4	924.7	882.1	989.9	916.7	930.7	42.4	4.6
phenanthrene	859.9	878.8	846.7	851.0	791.1	803.9	844.1	32.7	3.9
anthracene	991.0	919.5	1,045.6	1,075.7	1,235.5	1,133.4	1,047.9	117.2	11.2
fluoranthene	868.8	849.8	871.4	897.8	907.3	938.0	874.3	20.8	2.4
pyrene	789.2	755.4	808.0	820.5	827.2	899.6	795.0	26.5	3.3
benz[a]anthracene	801.6	885.8	812.8	841.9	760.3	865.3	815.1	45.2	5.6
chrysene	914.3	757.6	887.7	985.7	1,138.3	1,036.9	924.5	137.0	14.8
benzo[b]fluoranthene	792.0	857.1	868.6	848.3	749.1	714.1	816.7	48.7	6.0
benzo[k]fluoranthene	1,163.9	849.5	1,016.6	1,144.5	1,277.5	1,391.2	1,076.9	160.6	14.9
benzo[a]pyrene	950.7	859.9	874.1	960.7	913.4	956.1	899.5	35.5	3.9
indeno[123,cd]pyrene	860.3	844.6	828.8	892.3	874.8	868.0	852.1	17.2	2.0
dibenz[ah]anthracene	963.4	825.4	914.0	989.9	1,002.6	1,012.4	926.4	66.2	7.1
benzo[ghi]perylene	1,080.9	839.4	1,002.3	1,081.7	1,136.9	1,074.2	1,014.9	112.0	11.0
MEAN	924	852	897	930	958	960	907	61	6

[1] Nominal amount spiked in water column and SPMD spikes was ~ 900 ng per PAH.

TABLE X A. SPIKE RECOVERIES FOR WATER IN FRESHWATER STATIC EXPOSURES

PAHs	ng / Sample [1]								
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev.	Study C.V.
naphthalene	823	579	102[2]	<20[2]	<20[2]	785	729	107	15
acenaphthylene	865	759	281[2]	222[2]	228[2]	722	782	60	8
acenaphthene	856	768	312[2]	303[2]	278[2]	775	800	40	5
fluorene	861	861	391[2]	503[2]	526[2]	789	837	34	4
phenanthrene	870	784	651	789	829	834	793	70	9
anthracene	830	793	651	789	829	834	788	64	8
fluoranthene	927	867	749	1,001	1,082	1,019	941	110	12
pyrene	932	1,002	730	874	1,286	1,584	1,068	285	27
benz[a]anthracene	948	869	324	934	1,010	885	828	230	28
chrysene	1,052	1,016	324	934	---	885	842	266	32
benzo[b]fluorantheue	876	856	829	983	1,003	817	894	73	8
benzo[k]fluoranthene	885	849	829	983	1,003	817	894	73	8
benzo[a]pyrene	871	745	719	888	1,010	853	848	96	11
indeno[123,cd]pyrene	813	785	605	1,062	1,088	598	825	195	24
dibenz[ah]anthracene	759	729	535	736	828	424	668	141	21
benzo[ghi]perylene	668	694	461	642	749	537	625	97	16

[1] Sample = Amount in Spike Verification Table (IX.) added to 900 mL Control water and processed as water sample.
[2] = Low recovery of PAH due to loss, results not used in calculations.

TABLE X B. RECOVERY OF PAHs FROM SPIKED SPMD MEMBRANES FROM FRESHWATER STATIC EXPOSURES

PAHs [2]	ng / Membrane [1]									Mean ng/g
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Mean	Std Dev.	C.V.	
naphthalene	91	444	539	523	118	461	363	186	51.2	806
acenaphthylene	108	570	637	680	400	502	483	190	39.5	1,073
acenaphthene	155	523	608	651	429	536	484	163	33.6	1,075
fluoreue	163	577	654	626	410	570	500	169	33.9	1,111
phenanthrene	549	641	706	761	636	607	650	68	10.5	1,444
anthracene	549	641	706	761	636	607	650	68	10.5	1,444
fluoranthene	777	680	764	863	803	797	780	55	7.0	1,734
pyrene	833	680	828	877	793	821	805	61	7.6	1,790
benz[a]anthracene	876	752	793	912	753	745	805	66	8.2	1,789
chrysene	876	752	793	912	753	745	805	66	8.2	1,789
benzo[b]fluoranthene	851	697	742	904	747	776	786	70	9.0	1,747
benzo[k]fluoranthene	851	697	742	904	747	776	786	70	9.0	1,747
benzo[a]pyrene	839	672	742	961	826	805	807	89	11.0	1,794
indeno[123cd]pyrene	762	331	728	788	593	650	642	154	24.0	1,427
dibenz[ah]anthracene	822	585	746	806	546	768	712	107	15.0	1,583
benzo[ghi]perylene	747	594	624	901	752	704	720	100	13.9	1,601

[1] Membrane = 10 cm x 2.54 cm and 0.45g.
[2] Spike Verification table (IX) amounts spiked into SPMD membrane.

TABLE X C. RECOVERY OF PAHs FROM SPIKED SPMD LIPID FROM FRESHWATER STATIC EXPOSURES

PAHs [2]	ng / Lipid [1]									Mean ng/g
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Mean	Std Dev.	C.V.	
naphthalene	689	599	--- [3]	---	---	586	625	46	7.4	6,867
acenaphthylene	797	655	---	---	---	801	751	68	9.0	8,249
acenaphthene	838	655	---	---	---	816	769	82	10.6	8,455
fluorene	760	700	---	---	---	833	764	54	7.1	8,400
phenanthrene	843	739	---	---	---	841	808	49	6.0	8,874
anthracene	843	739	---	---	---	841	808	49	6.0	8,874
fluoranthene	867	755	---	---	---	924	849	70	8.3	9,325
pyrene	882	742	---	---	---	1,017	880	112	12.8	9,673
benz[a]anthracene	962	793	---	---	---	877	877	69	7.9	9,639
chrysene	962	793	---	---	---	877	877	69	7.9	9,639
benzo[b]fluoranthene	896	769	---	---	---	919	861	66	7.7	9,464
benzo[k]fluoranthene	896	769	---	---	---	919	861	66	7.7	9,464
benzo[a]pyrene	822	831	---	---	---	936	863	52	6.0	9,483
indeno[123cd]pyrene	850	691	---	---	---	757	766	65	8.5	8,418
dibenz[ah]anthracene	909	769	---	---	---	856	845	58	6.8	9,282
benzo[ghi]perylene	778	672	---	---	---	785	745	52	6.9	8,188

[1] Lipids = 0.1 mL (0.09g) triolein.
[2] Spike Verification table (IX) amounts spiked into SPMD lipid.
[3] --- = Samples losted during processing.

TABLE XI A. SPIKE RECOVERIES FOR WATER IN FRESHWATER STATIC EXPOSURES

PAHs	% RECOVERY								
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev.	Study C.V.
naphthalene	81	68	11[1]	0[1]	0[1]	87	78	8	10
acenaphthylene	96	90	33[1]	27[1]	26[1]	83	90	5	6
acenaphthene	88	89	35[1]	36[1]	31[1]	80	86	4	5
fluorene	99	92	42[1]	57[1]	53[1]	86	92	5	6
phenanthrene	101	89	77	93	105	104	95	10	10
anthracene	84	86	62	73	67	74	74	8	11
fluoranthene	107	102	86	112	119	109	106	10	10
pyrene	118	133	90	107	155	176	130	29	22
benz[a]anthracene	118	98	40	111	133	102	100	29	29
chrysene	115	134	37	95	---	85	93	33	35
benzo[b]fluoranthene	111	100	95	116	134	114	112	12	11
benzo[k]fluoranthene	76	100	82	86	79	59	80	12	15
benzo[a]pyrene	92	87	82	92	111	89	92	9	10
indeno[123,cd]pyrene	95	93	73	119	124	69	95	21	22
dibenz[ah]anthraceue	79	88	59	74	83	42	71	16	22
benzo[ghi]perylene	62	83	46	59	66	50	61	12	19
MEAN	95	96	59	79	80	88	91	14	15

[1] = Low recovery of PAH due to loss, results not used in calculations.

TABLE XI B. RECOVERY OF PAHs FROM SPIKED SPMD MEMBRANES FROM FRESHWATER STATIC EXPOSURES

PAHs	% Recovery [1]								
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev.	Study C.V.
naphthalene	9	53	59	59	13	50	55	4	7
acenaphthylene	15	69	69	83	42	59	70	9	12
acenaphthene	16	62	66	77	46	61	66	6	10
fluorene	18	61	74	76	47	63	69	7	10
phenanthrene	64	78	76	90	70	69	75	8	11
anthracene	64	78	76	90	70	69	75	8	11
fluoranthene	84	86	89	98	87	85	88	5	5
pyrene	90	81	94	101	87	83	89	7	8
benz[a]anthracene	99	87	98	100	87	79	92	8	8
chrysene	99	87	98	100	87	79	92	8	8
benzo[b]fluoranthene	96	87	88	99	84	83	90	6	7
benzo[k]fluoranthene	96	87	88	99	84	83	90	6	7
benzo[a]pyrene	100	82	84	102	89	83	90	8	9
indeno[123cd]pyrene	89	40	91	91	71	79	77	18	23
dibenz[ah]anthracene	86	73	85	88	58	79	78	10	13
benzo[ghi]perylene	80	71	77	127	82	93	88	19	21

[1] Spike Verification table (IX) amounts spiked into SPMD membrane.

TABLE XI C. RECOVERY OF PAHs FROM SPIKED SPMD LIPID FROM FRESHWATER STATIC EXPOSURES

PAHs	% Recovery [1]								
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev.	Study C.V.
naphthalene	71	72	--- [2]	---	---	63	69	4	5.7
acenaphthylene	114	80	---	---	---	94	96	14	14.7
acenaphthene	87	78	---	---	---	92	86	6	7.1
fluorene	83	74	---	---	---	91	83	7	8.4
phenanthrene	98	90	---	---	---	96	95	4	3.9
anthraceue	98	90	---	---	---	96	95	4	3.9
fluoranthene	94	95	---	---	---	99	96	2	2.2
pyrene	96	89	---	---	---	102	96	6	5.8
benz[a]anthracene	108	92	---	---	---	93	98	7	7.5
chrysene	108	92	---	---	---	93	98	7	7.5
benzo[b]fluoranthene	101	96	---	---	---	99	99	2	2.2
benzo[k]fluoranthene	101	96	---	---	---	99	99	2	2.2
benzo[a]pyrene	98	102	---	---	---	96	99	3	2.6
indeno[123cd]pyrene	99	84	---	---	---	92	92	6	6.8
dibenz[ah]anthracene	95	96	---	---	---	89	93	3	3.5
benzo[ghi]perylene	83	80	---	---	---	104	89	10	11.7

[1] Spike Verification table (IX) amounts spiked into SPMD lipid.

[2] --- = Samples losted during processing.

TABLE XII. PAH MDLs AND MQLs FOR 10 °C, 18 °C, & 26 °C FLOW-THROUGH DILUTER STUDY
(Standard Deviations of Lowest Calibration Standard, 0.05 ng/uL)

	ng / uL			
Compounds	Rep #1	Rep #2	Rep #3	Std Dev. [1]
naphthalene	0.0500	0.0474	0.0640	0.0083
acenaphthylene	0.0500	0.0519	0.0561	0.0021
acenaphthene	0.0500	0.0545	0.0629	0.0042
fluorene	0.0500	0.0380	0.0545	0.0082
phenanthrene	0.0500	0.0423	0.0501	0.0039
anthracene	0.0500	0.0533	0.0450	0.0041
fluoranthene	0.0500	0.0403	0.0377	0.0013
pyrene	0.0500	0.0280	0.0395	0.0057
benz[a]anthracene	0.0500	0.0401	0.0503	0.0051
chrysene	0.0500	0.0379	0.0381	0.0001
benzo[b]fluoranthene	0.0500	0.0425	0.0501	0.0038
benzo[k]fluoranthene	0.0500	0.0450	0.0437	0.0007
benzo[a]pyrene	0.0500	0.0430	0.0531	0.0050
indeno[123cd]pyrene	0.0500	0.0421	0.0540	0.0059
dibenz[ah]anthracene	0.0500	0.0423	0.0652	0.0114
benzo[ghi]perylene	0.0500	0.0431	0.0450	0.0010

[1] Standard Deviation used to calculate MDLs and MQLs of water and SPMD Control Samples.

TABLE XIII A. SPMD PROCESS BLANK FOR PAH 10 °C FLOW-THROUGH DILUTER STUDY

PAHs	ng / Blank [1]					
	Rep #1	Rep #2	Rep #3	Rep #4	Mean	Std Dev. [2]
naphthalene	<2 [3]	<2	<2	<2	<2	0.0083
acenaphthylene	<2	<2	<2	<2	<2	0.0021
acenaphthene	<2	<2	<2	<2	<2	0.0042
fluorene	<2	<2	<2	<2	<2	0.0082
phenanthrene	<2	<2	<2	<2	<2	0.0039
anthracene	<2	<2	<2	<2	<2	0.0041
fluoranthene	5	3	5	12	6	3.1302
pyrene	<2	<2	<2	<2	<2	0.0057
benz[a]anthracene	<2	<2	<2	<2	<2	0.0051
chrysene	<2	<2	<2	<2	<2	0.0001
benzo[b]fluoranthene	<2	<2	<2	<2	<2	0.0038
benzo[k]fluoranthene	<2	<2	<2	<2	<2	0.0007
benzo[a]pyrene	2	2	2	2	2	0.1732
indeno[123cd]pyrene	<2	<2	<2	<2	<2	0.0059
dibenz[ah]anthracene	<2	<2	<2	<2	<2	0.0114
benzo[ghi]perylene	<2	<2	<2	<2	<2	0.0010

[1] Blank = Dialysis solvent processed as SPMD.

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XIII B. SPMD PROCESS BLANK FOR PAH 18 °C FLOW-THROUGH DILUTER STUDY

PAHs	ng / Blank [1]					
	Rep #5	Rep #6	Rep #7	Rep #8	Mean	Std Dev. [2]
naphthalene	2	17	2	2	6	6.5385
acenaphthylene	2	6	2	2	3	1.9053
acenaphthene	<2 [3]	<2	<2	<2	<2	0.0042
fluorene	<2	<2	<2	<2	<2	0.0082
phenanthrene	<2	<2	<2	<2	<2	0.0039
anthracene	<2	<2	<2	<2	<2	0.0041
fluoranthene	2	5	2	5	4	1.2952
pyrene	<2	<2	<2	<2	<2	0.0057
benz[a]anthracene	<2	<2	<2	<2	<2	0.0051
chrysene	<2	<2	<2	<2	<2	0.0001
benzo[b]fluoranthene	<2	<2	<2	<2	<2	0.0038
benzo[k]fluoranthene	<2	<2	<2	<2	<2	0.0007
benzo[a]pyrene	<2	<2	<2	<2	<2	0.0050
indeno[123cd]pyrene	<2	<2	<2	<2	<2	0.0059
dibenz[ah]anthracene	<2	<2	<2	<2	<2	0.0114
benzo[ghi]perylene	<2	<2	<2	<2	<2	0.0010

[1] Blank = Dialysis solvent processed as SPMD.

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XIII C. SPMD PROCESS BLANK FOR PAH 26 °C FLOW-THROUGH DILUTER STUDY

PAHs	ng / Blank [1]					
	Rep #9	Rep #10	Rep #11	Rep #12	Mean	Std Dev. [2]
naphthalene	<2 [3]	<2	<2	<2	<2	0.0083
acenaphthylene	<2	<2	<2	<2	<2	0.0021
acenaphthene	<2	<2	<2	<2	<2	0.0042
fluorene	<2	<2	<2	<2	<2	0.0082
phenanthrene	<2	<2	<2	<2	<2	0.0039
anthracene	<2	<2	<2	<2	<2	0.0041
fluoranthene	39	9	2	8	14	12.9450
pyrene	2	46	2	2	13	18.9227
benz[a]anthracene	<2	<2	<2	<2	<2	0.0051
chrysene	<2	<2	<2	<2	<2	0.0001
benzo[b]fluoranthene	<2	<2	<2	<2	<2	0.0038
benzo[k]fluoranthene	<2	<2	<2	<2	<2	0.0007
benzo[a]pyrene	<2	<2	<2	<2	<2	0.0051
indeno[123cd]pyrene	<2	<2	<2	<2	<2	0.0059
dibenz[ah]anthracene	<2	<2	<2	<2	<2	0.0114
benzo[ghi]perylene	<2	<2	<2	<2	<2	0.0010

[1] Blank = Dialysis solvent processed as SPMD.

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XIV A. PAH 10 °C FLOW-THROUGH DILUTER STUDY (SPMD Dialysis Blank)

PAH	ng / SPMD [1]					
	Rep #1	Rep #2	Rep #3	Rep #4	Mean	Std Dev. [2]
naphthalene	22	17	18	26	21	3.351
acenaphthylene	<2 [3]	<2	<2	<2	<2	0.002
acenaphthene	<2	<2	<2	<2	<2	0.004
fluorene	3	4	4	3	4	0.453
phenanthrene	2	2	7	7	5	2.525
anthracene	<2	<2	<2	<2	<2	0.004
fluoranthene	3	2	2	27	9	9.361
pyrene	2	3	3	7	4	1.761
benz[a]anthracene	<2	<2	<2	<2	<2	0.005
chrysene	<2	<2	<2	<2	<2	0.000
benzo[b]fluoranthene	<2	<2	<2	<2	<2	0.004
benzo[k]fluoranthene	<2	<2	<2	<2	<2	0.001
benzo[a]pyrene	<2	<2	<2	<2	<2	0.005
indeno[123cd]pyrene	2	2	2	9	4	2.634
dibenz[ah]anthracene	2	2	2	4	3	0.852
benzo[ghi]perylene	2	2	2	4	3	0.775

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XIV B. PAH 18 °C FLOW-THROUGH DILUTER STUDY
(SPMD Dialysis Blank)

PAH	ng / SPMD [1]					
	Rep #5	Rep #6	Rep #7	Rep #8	Mean	Std Dev. [2]
naphthalene	33	70	38	8	37	22.257
acenaphthylene	<2 [3]	<2	<2	<2	<2	0.002
acenaphthene	<2	<2	<2	<2	<2	0.004
fluorene	5	6	2	2	4	1.571
phenanthrene	10	6	6	2	6	2.935
anthracene	6	2	2	2	3	1.627
fluoranthene	117	5	2	4	32	43.724
pyrene	158	5	3	2	42	59.990
benz[a]anthracene	<2	<2	<2	<2	<2	0.005
chrysene	2	13	2	2	5	4.222
benzo[b]fluoranthene	3	2	2	2	2	0.387
benzo[k]fluoranthene	<2	<2	<2	<2	<2	0.001
benzo[a]pyrene	4	2	2	2	3	0.909
indeno[123cd]pyrene	24	2	2	2	7	8.366
dibenz[ah]anthracene	2	2	2	2	2	0.116
benzo[ghi]perylene	5	2	2	2	3	1.239

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).
[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).
[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XIV C. PAH 26 °C FLOW-THROUGH DILUTER STUDY (SPMD Dialysis Blank)

PAH	ng / SPMD [1]					
	Rep #9	Rep #10	Rep #11	Rep #12	Mean	Std Dev. [2]
naphthalene	17	39	42	5	26	15.402
acenaphthylene	<2 [3]	<2	<2	<2	<2	0.002
acenaphthene	<2	<2	<2	<2	<2	0.004
fluorene	2	2	2	4	2	0.608
phenanthrene	4	3	7	5	5	1.257
anthracene	<2	<2	<2	<2	<2	0.004
fluoranthene	3	2	2	10	4	2.976
pyrene	2	2	5	28	9	10.952
benz[a]anthracene	<2	<2	<2	<2	<2	0.005
chrysene	<2	<2	<2	<2	<2	0.000
benzo[b]fluoranthene	<2	<2	<2	<2	<2	0.004
benzo[k]fluoranthene	<2	<2	<2	<2	<2	0.001
benzo[a]pyrene	2	2	2	11	4	3.524
indeno[123cd]pyrene	2	2	2	8	3	2.468
dibenz[ah]anthracene	<2	<2	<2	<2	<2	0.011
benzo[ghi]perylene	<2	<2	<2	<2	<2	0.001

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XV A. PAH 10 °C FLOW-THROUGH DILUTER STUDY (SPMD Storage Blank)

PAHs	ng / SPMD [1]				
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]
naphthalene	25	17	27	23	4.1492
acenaphthylene	<2 [3]	<2	<2	<2	0.0021
acenaphthene	<2	<2	<2	<2	0.0042
fluorene	3	3	4	3	0.3082
phenanthrene	6	5	5	5	0.5907
anthracene	<2	<2	<2	<2	0.0041
fluoranthene	2	2	6	3	1.5513
pyrene	2	3	2	2	0.4143
benz[a]anthracene	<2	<2	<2	<2	0.0051
chrysene	<2	<2	<2	<2	0.0001
benzo[b]fluoranthene	<2	<2	<2	<2	0.0038
benzo[k]fluoranthene	<2	<2	<2	<2	0.0007
benzo[a]pyrene	<2	<2	<2	<2	0.0050
indeno[123cd]pyrene	<2	<2	<2	<2	0.0059
dibenz[ah]anthracene	<2	<2	<2	<2	0.0114
benzo[ghi]perylene	<2	<2	<2	<2	0.0010

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XV B. PAH 18 °C FLOW-THROUGH DILUTER STUDY (SPMD Storage Blank)

PAHs	ng / SPMD [1]				
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]
naphthalene	20	18	15	18	2.3697
acenaphthylene	<2 [3]	<2	<2	<2	0.0021
acenaphthene	<2	<2	<2	<2	0.0042
fluorene	2	2	3	2	0.1871
phenanthrene	8	8	7	8	0.3300
anthracene	<2	<2	<2	<2	0.0041
fluoranthene	31	6	5	14	10.5739
pyrene	6	2	3	4	1.5006
benz[a]anthracene	<2	<2	<2	<2	0.0051
chrysene	2	10	2	5	3.2660
benzo[b]fluoranthene	<2	<2	<2	<2	0.0038
benzo[k]fluoranthene	<2	<2	<2	<2	0.0007
benzo[a]pyrene	<2	<2	<2	<2	0.0050
indeno[123cd]pyrene	2	5	2	3	1.2656
dibenz[ah]anthracene	<2	<2	<2	<2	0.0114
benzo[ghi]perylene	<2	<2	<2	<2	0.0010

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XV C. PAH 26 °C FLOW-THROUGH DILUTER STUDY (SPMD Storage Blank)

PAHs	ng / SPMD [1]				
	Rep #1	Rep #2	Rep #3	Mean	Std Dev. [2]
naphthalene	29	43	34	35	5.8903
acenaphthylene	<2 [3]	<2	<2	<2	0.0021
acenaphthene	<2	<2	<2	<2	0.0042
flnorene	2	3	4	3	0.5788
phenanthrene	10	7	8	8	1.0339
anthracene	<2	<2	<2	<2	0.0041
fluoranthene	11	2	4	6	3.3459
pyrene	10	3	6	6	2.2924
benz[a]anthracene	<2	<2	<2	<2	0.0051
chrysene	<2	<2	<2	<2	0.0001
benzo[b]fluoranthene	<2	<2	<2	<2	0.0038
benzo[k]fluoranthene	<2	<2	<2	<2	0.0007
benzo[a]pyrene	<2	<2	<2	<2	0.0050
indeno[123cd]pyrene	<2	<2	<2	<2	0.0059
dibenz[ah]anthracene	<2	<2	<2	<2	0.0114
benzo[ghi]perylene	<2	<2	<2	<2	0.0010

[1] SPMDs = 45.7 cm x 2.54 cm with 0.5 mL (0.455g) triolein (Total SPMD mass = 2.48g).

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XVI A. SPIKE RECOVERIES FOR 10 °C PAH FLOW-THROUGH DILUTER EXPOSURES (GPC Spike for SPMDs)

PAHs	Spike Verification ng	ng/Sample [1]							Mean ng/g
		Rep 1	Rep 2	Rep 3	Rep 4	Mean	Std. Dev.	C.V.	
naphthalene	2,401	1,287.2	1,603.5	1,830.3	1,655.4	1,594.1	196.1	12.3	643
acenaphthylene	2,314	1,412.3	1,749.8	1,842.5	1,745.0	1,687.4	163.5	9.7	680
acenaphthene	2,314	1,377.2	1,737.2	1,907.2	1,740.6	1,690.6	193.5	11.4	682
fluorene	2,376	1,849.8	1,896.1	1,908.9	1,939.7	1,898.6	32.3	1.7	766
phenanthrene	3,180	2,010.1	1,669.6	1,755.0	1,681.3	1,779.0	137.4	7.7	717
anthracene	1,854	1,981.9	1,831.4	2,040.6	1,906.2	1,940.0	78.8	4.1	782
fluoranthene	2,432	2,237.7	1,972.5	2,051.9	1,971.2	2,058.3	108.6	5.3	830
pyrene	2,627	2,150.6	1,894.3	2,067.7	2,046.8	2,039.9	92.6	4.5	823
benz[a]anthracene	2,259	2,389.6	1,746.9	1,725.6	1,840.3	1,925.6	271.3	14.1	776
chrysene	2,300	2,508.5	1,932.1	1,975.9	2,033.2	2,112.4	231.5	11.0	852
benzo[b]fluoranthene	2,171	2,268.3	1,741.5	1,612.3	1,813.7	1,859.0	247.1	13.3	750
benzo[k]fluoranthene	2,358	3,121.3	1,885.1	1,998.1	1,968.5	2,243.3	508.6	22.7	905
benzo[a]pyrene	2,169	2,573.3	1,829.1	1,821.8	1,874.5	2,024.7	317.4	15.7	816
indeno[123,cd]pyrene	2,143	2,443.1	1,751.2	1,737.4	1,862.7	1,948.6	289.6	14.9	786
dibenz[ah]anthracene	2,242	2,328.7	1,179.7	1,075.9	1,899.8	1,621.0	517.3	31.9	654
benzo[ghi]perylene	2,159	2,107.6	1,527.0	1,482.6	1,865.1	1,745.6	256.1	14.7	704

[1] Samples = Amount in Spike Verification column added to solvents and taken through SPMD GPC process.

TABLE XVI B. SPIKE RECOVERIES FOR 18 °C PAH FLOW-THROUGH DILUTER EXPOSURES (GPC Spike for SPMDs)

PAHs	Spike Verification ng	ng/Sample [1]							Mean ng/g
		Rep 1	Rep 2	Rep 3	Rep 4	Mean	Std. Dev.	C.V.	
naphthalene	2,401	1,345.4	1,721.8	1,791.2	1,545.5	1,601.0	172.6	10.8	646
acenaphthylene	2,314	1,546.2	1,597.0	1,698.8	1,604.2	1,611.6	55.1	3.4	650
acenaphthene	2,314	1,501.2	1,505.9	1,616.4	1,483.7	1,526.8	52.4	3.4	616
fluorene	2,376	1,959.1	1,632.9	1,660.7	1,608.4	1,715.3	142.0	8.3	692
phenanthrene	3,180	1,632.1	1,536.1	1,613.0	1,569.5	1,587.7	37.4	2.4	640
anthracene	1,854	2,006.2	1,920.9	1,807.3	1,919.2	1,913.4	70.6	3.7	772
fluoranthene	2,432	2,022.1	1,844.2	1,854.8	1,706.7	1,857.0	111.8	6.0	749
pyrene	2,627	2,217.0	1,662.7	1,560.2	1,571.3	1,752.8	270.9	15.5	707
benz[a]anthracene	2,259	1,783.9	1,566.8	1,552.7	1,495.1	1,599.6	109.7	6.9	645
chrysene	2,300	2,033.2	1,534.6	1,715.3	1,766.7	1,762.5	178.5	10.1	711
benzo[b]fluoranthene	2,171	1,698.1	1,646.9	1,576.2	1,738.4	1,664.9	60.6	3.6	671
benzo[k]fluoranthene	2,358	2,107.3	1,820.8	1,806.5	1,919.7	1,913.6	120.0	6.3	772
benzo[a]pyrene	2,169	1,826.0	1,795.4	1,713.9	1,858.7	1,798.5	53.7	3.0	725
indeno[123,cd]pyrene	2,143	1,853.8	1,803.5	1,549.5	1,728.6	1,733.9	115.4	6.7	699
dibenz[ah]anthracene	2,242	1,741.9	1,933.3	1,742.1	1,862.9	1,820.1	81.9	4.5	734
benzo[ghi]perylene	2,159	1,838.7	1,695.0	1,495.2	1,744.0	1,693.2	125.5	7.4	683

[1] Samples = Amount in Spike Verification column added to solvents and taken through SPMD GPC process.

TABLE XVI C. SPIKE RECOVERIES FOR 26 °C PAH FLOW-THROUGH DILUTER EXPOSURES (GPC Spike for SPMDs)

PAHs	Spike Verification ng	ng/Sample [1]							Mean ng/g
		Rep 1	Rep 2	Rep 3	Rep 4	Mean	Std. Dev.	C.V.	
naphthalene	2,401	2,044.5	1,959.3	1,987.0	1,813.1	1,951.0	85.3	4.4	787
acenaphthylene	2,314	1,889.2	1,779.0	1,904.2	1,697.2	1,817.4	84.6	4.7	733
acenaphthene	2,314	1,873.7	1,769.1	1,837.3	1,690.9	1,792.8	69.8	3.9	723
fluorene	2,376	1,576.6	1,440.5	1,507.5	1,414.1	1,484.7	63.1	4.2	599
phenanthrene	3,180	1,626.1	1,680.1	1,844.0	1,534.0	1,671.1	112.7	6.7	674
anthracene	1,854	2,031.6	1,829.2	1,843.3	1,646.7	1,837.7	136.2	7.4	741
fluoranthene	2,432	1,737.9	1,838.5	1,869.4	1,773.4	1,804.8	51.9	2.9	728
pyrene	2,627	1,690.8	1,828.7	1,745.8	1,438.0	1,675.8	145.8	8.7	676
benz[a]anthracene	2,259	1,642.9	1,669.7	1,607.6	1,482.1	1,600.6	71.9	4.5	645
chrysene	2,300	1,883.4	1,761.1	1,872.4	1,675.2	1,798.0	85.5	4.8	725
benzo[b]fluoranthene	2,171	1,231.7	1,424.8	1,413.3	1,580.9	1,412.7	123.7	8.8	570
benzo[k]fluoranthene	2,358	2,237.2	2,222.8	2,210.8	1,823.5	2,123.6	173.5	8.2	856
benzo[a]pyrene	2,169	1,911.4	1,715.1	1,915.5	1,716.0	1,814.5	99.0	5.5	732
indeno[123,cd]pyrene	2,143	1,586.9	1,530.6	1,645.8	1,364.6	1,532.0	104.9	6.8	618
dibenz[ah]anthracene	2,242	1,838.0	1,815.6	1,774.1	1,478.6	1,726.6	145.0	8.4	696
benzo[ghi]perylene	2,159	1,479.8	1,544.2	1,595.8	1,481.6	1,525.4	48.2	3.2	615

[1] Samples = Amount in Spike Verification column added to solvents and taken through SPMD GPC process.

TABLE XVI D. SPIKE RECOVERIES FOR PAH FLOW-THROUGH DILUTER EXPOSURES
(GPC Spike for SPMDs Summary)

PAHs	% Recovery			
	10 °C Mean	18 °C Mean	26 °C Mean	Study Mean
naphthalene	66	67	81	71
acenaphthylene	73	70	79	74
acenaphthene	73	66	77	72
fluorene	80	72	62	72
phenanthrene	56	50	53	53
anthracene	105	103	99	102
fluoranthene	85	76	74	78
pyrene	78	67	64	69
benz[a]anthracene	85	71	71	76
chrysene	92	77	78	82
benzo[b]fluoranthene	86	77	65	76
benzo[k]fluoranthene	95	81	90	89
benzo[a]pyrene	93	83	84	87
indeno[123,cd]pyrene	91	81	71	81
dibenz[ah]anthracene	72	81	77	77
benzo[ghi]perylene	81	78	71	77

TABLE XVII A. WATER MDLs AND MQLs FOR PAH FLOW-THROUGH DILUTER STUDY
(Control Water Data)

PAHs	ng / 8L Sample [1]								
	10 °C Day 0	10 °C Day 21	10 °C Average	18 °C Day 0	18 °C Day 21	18 °C Average	26 °C Day 0	26 °C Day 21	26 °C Average
naphthalene	10	2	6	<2 [2]	<2	<2	15	10	12
acenaphthylene	<2	<2	<2	<2	<2	<2	<2	<2	<2
acenaphthene	<2	<2	<2	<2	<2	<2	2	4	3
fluorene	8	2	5	<2	<2	<2	<2	<2	<2
phenanthrene	11	6	8	4	2	3	4	5	5
anthracene	2	2	2	10	2	6	2	2	2
fluoranthene	7	4	5	7	2	5	31	7	19
pyrene	10	8	9	6	2	4	2	4	3
benz[a]anthracene	<2	<2	<2	<2	<2	<2	<2	<2	<2
chrysene	<2	<2	<2	<2	<2	<2	<2	<2	<2
benzo[b]fluoranthene	<2	<2	<2	<2	<2	<2	<2	<2	<2
benzo[k]fluoranthene	<2	<2	<2	<2	<2	<2	6	2	4
benzo[a]pyrene	3	2	2	10	2	6	16	11	14
indeno[123cd]pyrene	<2	<2	<2	<2	<2	<2	<2	<2	<2
dibenz[ah]anthracene	<2	<2	<2	12	2	7	<2	<2	<2
benzo[ghi]perylene	<2	<2	<2	<2	<2	<2	<2	<2	<2

[1] Sample = 8 L of Control Chamber water.
[2] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XVII B. WATER MDLs AND MQLs FOR PAH FLOW-THROUGH DILUTER STUDY
(Control Water Study Summary)

PAHs	ng / 8L Sample [1]					Study Mean ng/L
	Study Mean	Study Std Dev. [2]	C.V.	Study MDL	Study MQL	
naphthalene	7	4.302	63	20	50	0.9
acenaphthylene	<2 [3]	0.002	---	2	2	<0.3
acenaphthene	2	0.401	18	3	6	0.3
fluorene	3	1.485	49	8	18	0.4
phenanthrene	5	2.147	40	12	27	0.7
anthracene	3	1.886	57	9	22	0.4
fluoranthene	10	6.537	68	29	75	1.2
pyrene	5	2.797	53	14	33	0.7
benz[a]anthracene	<2	0.005	---	2	2	<0.3
chrysene	<2	0.000	---	2	2	<0.3
benzo[b]fluoranthene	<2	0.004	---	2	2	<0.3
benzo[k]fluoranthene	3	0.849	33	5	11	0.3
benzo[a]pyrene	7	4.699	65	21	54	0.9
indeno[123cd]pyrene	<2	0.006	---	2	2	<0.3
dibenz[ah]anthracene	4	2.357	64	11	27	0.5
benzo[ghi]perylene	<2	0.001	---	2	2	<0.3

[1] Sample = 8 L of Control Chamber water.

[2] Staudard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XVIII A. WATER PROCESS BLANKS FOR PAH FLOW-THROUGH DILUTER STUDY

PAHs	ng / Blank [1]								
	10 °C Day 0	10 °C Day 21	10 °C Average	18 °C Day 0	18 °C Day 21	18 °C Average	26 °C Day 0	26 °C Day 21	26 °C Average
naphthalene	<2 [2]	<2	<2	3	2	3	6	2	4
acenaphthylene	<2	<2	<2	<2	<2	<2	<2	<2	<2
acenaphthene	<2	<2	<2	<2	<2	<2	<2	<2	<2
fluorene	<2	<2	<2	<2	<2	<2	<2	<2	<2
phenanthrene	<2	<2	<2	<2	<2	<2	<2	<2	<2
anthracene	<2	<2	<2	3	2	3	3	2	2
fluoranthene	<2	<2	<2	<2	<2	<2	2	6	4
pyrene	5	2	3	2	18	10	<2	<2	<2
benz[a]anthracene	<2	<2	<2	<2	<2	<2	<2	<2	<2
chrysene	<2	<2	<2	<2	<2	<2	<2	<2	<2
benzo[b]fluoranthene	<2	<2	<2	<2	<2	<2	<2	<2	<2
benzo[k]fluoranthene	<2	<2	<2	<2	<2	<2	<2	<2	<2
benzo[a]pyrene	3	2	2	2	14	8	2	3	3
indeno[123cd]pyrene	3	2	3	35	2	18	2	2	2
dibenz[ah]anthracene	<2	<2	<2	<2	<2	<2	<2	<2	<2
benzo[ghi]perylene	<2	<2	<2	<2	<2	<2	<2	<2	<2

[1] Blanks = Extraction solvent processed as water samples.

[2] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XVIII B. WATER PROCESS BLANKS FOR PAH FLOW-THROUGH DILUTER STUDY (Study Summary)

PAHs	ng / Blank [1]		
	Study Mean	Study Std Dev. [2]	C.V.
naphthalene	3	0.811	28
acenaphthylene	<2 [3]	0.002	---
acenaphthene	<2	0.004	---
fluorene	<2	0.008	---
phenanthrene	<2	0.004	---
anthracene	2	0.227	10
fluoranthene	3	1.014	37
pyrene	5	3.434	67
benz[a]anthracene	<2	0.005	---
chrysene	<2	0.000	---
benzo[b]fluoranthene	<2	0.004	---
benzo[k]fluoranthene	<2	0.001	---
benzo[a]pyrene	4	2.668	61
indeno[123cd]pyrene	8	7.534	99
dibenz[ah]anthracene	<2	0.011	---
benzo[ghi]perylene	<2	0.001	---

[1] Blanks = Extraction solvent processed as water samples.

[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XII).

[3] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XIX A. SPIKE RECOVERIES FOR PAH FLOW-THROUGH DILUTER EXPOSURES
(Potassium Silicate Column Spike for Water)

PAHs	Spike Verification ng	ng/Sample [1]						
		Rep 1	Rep 2	Rep 3	Rep 4	Mean	Std. Dev	C.V.
naphthalene	195	69	149	143	140	125	33	26
acenaphthylene	184	100	160	155	175	147	28	19
acenaphthene	188	114	167	167	165	153	23	15
fluorene	144	142	176	157	168	160	13	8
phenanthrene	158	152	166	166	155	160	6	4
anthracene	188	153	174	185	190	175	14	8
fluoranthene	156	164	162	154	172	163	6	4
pyrene	152	154	153	137	165	152	10	7
benz[a]anthracene	168	179	181	187	201	187	9	5
chrysene	174	180	193	154	179	176	14	8
benzo[b]fluoranthene	198	182	194	189	211	194	11	6
benzo[k]fluoranthene	200	200	181	162	174	179	14	8
benzo[a]pyrene	195	177	177	184	191	182	6	3
indeno[123,cd]pyrene	200	188	173	206	202	192	13	7
dibenz[ah]anthracene	220	186	184	186	212	192	11	6
benzo[ghi]perylene	226	184	176	184	225	192	19	10

[1] Sample = Amount in Spike Verification column added to Potassium Silicate column and processed as water sample.

TABLE XIX B. SPIKE RECOVERIES (PERCENT) FOR PAH FLOW-THROUGH DILUTER EXPOSURES
(Potassium Silicate Column Spike for Water)

PAHs	Study % Recovery
naphthalene	64.3
acenaphthylene	80.2
acenaphthene	81.7
fluorene	111.4
phenanthrene	101.6
anthracene	93.1
fluoranthene	104.7
pyrene	100.2
benz[a]anthracene	111.0
chrysene	101.5
benzo[b]fluoranthene	98.1
benzo[k]fluoranthene	89.5
benzo[a]pyrene	93.2
indeno[123,cd]pyrene	96.2
dibenz[ah]anthracene	87.1
benzo[ghi]perylene	85.0

TABLE XX. WATER MDLs and MQLs FOR PAH FRESHWATER STATIC EXPOSURES
(Standard Deviations of Lowest Calibration Standard, 0.1 ng/uL)

PAHs	ng / uL				
	Rep #1	Rep #2	Rep #3	Stv Dev.[1]	C.V.
naphthalene	0.100	0.102	0.101	0.001	1
acenaphthylene	0.100	0.092	0.087	0.006	6
acenaphthene	0.100	0.096	0.100	0.002	2
fluorene	0.100	0.093	0.163	0.032	19
phenanthrene	0.100	0.094	0.108	0.006	5
anthracene	0.100	0.094	0.111	0.007	6
fluoranthene	0.100	0.092	0.095	0.003	3
pyrene	0.100	0.109	0.107	0.004	3
benz[a]anthracene	0.100	0.096	0.106	0.004	4
chrysene	0.100	0.096	0.106	0.004	4
benzo[b]fluoranthene	0.100	0.094	0.104	0.004	4
benzo[k]fluoranthene	0.100	0.094	0.104	0.004	4
benzo[a]pyrene	0.100	0.094	0.117	0.010	8
indeno[123cd]pyrene	0.100	0.098	0.075	0.012	15
dibenz[ah]anthracene	0.100	0.097	0.100	0.001	1
benzo[ghi]perylene	0.100	0.106	0.135	0.015	11

[1] Standard Deviation used to calculate MDLs and MQLs of water and SPMD Control Samples.

TABLE XXI A. SPMD MEMBRANE PROCESS BLANK FOR PAH FRESHWATER STATIC EXPOSURES

PAHs	ng / Blank [1]										Study Mean ng/g
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev. [2]	Study MDL	Study MQL	
naphthalene	<10 [3]	<10	<10	<10	<10	10	10	0.001	10	10	4.9
acenaphthylene	<10	<10	<10	<10	<10	<10	<10	0.006	<10	<10	<4.9
acenaphthene	<10	<10	<10	<10	<10	<10	<10	0.002	<10	<10	<4.9
fluorene	<10	<10	12	<10	<10	<10	10	0.032	10	11	5.1
phenanthrene	<10	<10	<10	<10	<10	48	16	0.006	<10	<10	8.0
anthracene	<10	<10	<10	<10	<10	48	16	0.007	<10	<10	8.0
fluoranthene	25	<10	<10	<10	41	265	60	92.395	337	984	29.7
pyrene	79	<10	21	23	170	669	162	233.145	861	2,493	80.0
benz[a]anthracene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<4.9
chrysene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<4.9
benzo[b]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<4.9
benzo[k]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<4.9
benzo[a]pyrene	<10	<10	<10	<10	<10	<10	<10	0.010	<10	<10	<4.9
indeno[123cd]pyrene	<10	<10	<10	<10	<10	55	17	0.012	18	18	8.6
dibenz[ah]anthracene	<10	<10	<10	<10	<10	<10	<10	0.001	<10	<10	<4.9
benzo[ghi]perylene	<10	<10	<10	<10	<10	<10	<10	0.015	<10	<10	<4.9

[1] Blank = Dialysis solvent processed as SPMD membrane.
[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XX).
[3] < = Background or samples with values less than the average noise peak area of 1,000 or 10 ng/0.5 mL sample.

TABLE XXI B. SPMD LIPID PROCESS BLANK FOR PAH FRESHWATER STATIC EXPOSURES

PAHs	ng / Blank [1]										Study Mean ng/g
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56	Study Mean	Study Std Dev. [2]	Study MDL	Study MQL	
naphthalene	<10 [3]	<10	<10	<10	<10	<10	<10	0.001	<10	<10	<22
acenaphthylene	<10	<10	<10	<10	<10	<10	<10	0.006	<10	<10	<22
acenaphthene	<10	<10	<10	<10	<10	<10	<10	0.002	<10	<10	<22
fluorene	<10	33	<10	<10	<10	<10	14	0.032	14	14	30
phenanthrene	<10	<10	<10	<10	<10	<10	<10	0.006	<10	<10	<22
anthracene	<10	<10	<10	<10	<10	<10	<10	0.007	<10	<10	<22
fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.003	<10	<10	<22
pyrene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
benz[a]anthracene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
chrysene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
benzo[b]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
benzo[k]fluoranthene	<10	<10	<10	<10	<10	<10	<10	0.004	<10	<10	<22
benzo[a]pyrene	<10	<10	<10	<10	<10	<10	<10	0.010	<10	<10	<22
indeno[123cd]pyrene	<10	<10	<10	<10	<10	<10	<10	0.012	<10	<10	<22
dibenz[ah]anthracene	<10	<10	<10	<10	<10	<10	<10	0.001	<10	<10	<22
benzo[ghi]perylene	<10	41	<10	<10	<10	<10	15	0.015	15	15	33

[1] Blank = Dialysis solvent processed as SPMD membrane.
[2] Standard Deviation of samples with values having a prefix of < were derived from the Standard Deviation of the lowest Calibration Standard (Table XX).
[3] < = Background or samples with values less than the average noise peak area of 1,000 or 10 ng/0.5 mL sample.

TABLE XXII A. WATER MDLs and MQLs FOR PAH FRESHWATER STATIC EXPOSURES
(Control Water Data)

PAHs	ng / Sample [1]					
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56
naphthalene	<2 [2]	<2	<2	<2	3	<2
acenaphthylene	<2	<2	<2	<2	<2	<2
acenaphthene	<2	<2	<2	<2	<2	<2
fluorene	<2	<2	<2	<2	<2	<2
phenanthrene	<2	<2	<2	<2	<2	<2
anthracene	<2	<2	<2	<2	<2	<2
fluoranthene	<2	<2	<2	<2	<2	15
pyrene	<2	<2	<2	<2	<2	49
benz[a]anthracene	<2	<2	<2	<2	17	<2
chrysene	<2	<2	<2	<2	17	<2
benzo[b]fluoranthene	<2	<2	<2	<2	<2	<2
benzo[k]fluoranthene	<2	<2	<2	<2	<2	<2
benzo[a]pyrene	<2	<2	<2	<2	<2	<2
indeno[123cd]pyrene	<2	<2	<2	<2	<2	<2
dibenz[ah]anthracene	<2	<2	<2	<2	<2	<2
benzo[ghi]perylene	<2	<2	<2	9	<2	<2

[1] Sample = 8 L of Control Chamber water.

[2] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XXII B. WATER MDLs and MQLs FOR PAH FRESHWATER STATIC EXPOSURES
(Control Water Study Summary)

PAHs	ng / Sample [1]				Study Mean ng/L
	Study Mean	Study Stv Dev.[3]	Study MDL	Study MQL	
naphthalene	2	0.001	2	2	2.4
acenaphthylene	<2 [2]	0.006	<2	<2	<2
acenaphthene	<2	0.002	<2	<2	<2
fluorene	<2	0.032	<2	<2	<2
phenanthrene	<2	0.006	<2	<2	<2
anthracene	<2	0.007	<2	<2	<2
fluoranthene	4	0.003	4	4	4.6
pyrene	10	0.004	10	10	11.0
benz[a]anthracene	5	0.004	5	5	5.1
chrysene	5	0.004	5	5	5.1
benzo[b]fluoranthene	<2	0.004	<2	<2	<2
benzo[k]fluoranthene	<2	0.004	<2	<2	<2
benzo[a]pyrene	<2	0.010	<2	<2	<2
indeno[123cd]pyrene	<2	0.012	<2	<2	<2
dibenz[ah]anthracene	<2	0.001	<2	<2	<2
benzo[ghi]perylene	3	0.015	3	3	3.6

[1] Sample = 8 L of Control Chamber water.

[2] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

[3] Standard Deviation of samples were derived from the Standard Deviation of lowest Calibtation Standard (Table XX).

TABLE XXIII A. WATER PROCESS BLANKS FOR PAH FRESHWATER STATIC EXPOSURES

PAHs	ng / Blank [1]					
	Day 1	Day 4	Day 7	Day 14	Day 28	Day 56
naphthalene	<2 [2]	<2	<2	<2	<2	<2
acenaphthylene	<2	<2	<2	<2	<2	<2
acenaphthene	<2	<2	<2	<2	<2	<2
fluorene	<2	<2	<2	<2	<2	<2
phenanthrene	<2	<2	<2	<2	<2	<2
anthracene	<2	<2	<2	<2	<2	<2
fluoranthene	<2	<2	<2	<2	<2	6
pyrene	<2	<2	<2	<2	3	32
benz[a]anthracene	<2	<2	<2	<2	12	<2
chrysene	<2	<2	<2	<2	12	<2
benzo[b]fluoranthene	<2	<2	<2	<2	<2	<2
benzo[k]fluoranthene	<2	<2	<2	<2	<2	<2
benzo[a]pyrene	<2	11	<2	<2	33	<2
indeno[123cd]pyrene	15	<2	<2	<2	<2	<2
dibenz[ah]anthracene	<2	<2	<2	<2	<2	<2
benzo[ghi]perylene	<2	<2	10	<2	34	<2

[1] Blanks = Extraction solvent processed as water samples.

[2] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

TABLE XXIII B. WATER PROCESS BLANKS FOR PAH FRESHWATER STATIC EXPOSURES (Study Summary)

PAHs	ng / Blank [1]	
	Study Mean	Study Stv Dev.[3]
naphthalene	<2 [2]	0.001
acenaphthylene	<2	0.006
acenaphthene	<2	0.002
fluorene	<2	0.032
phenanthrene	<2	0.006
anthracene	<2	0.007
fluoranthene	3	0.003
pyrene	7	0.004
benz[a]anthracene	4	0.004
chrysene	4	0.004
benzo[b]fluoranthene	<2	0.004
benzo[k]fluoranthene	<2	0.004
benzo[a]pyrene	9	0.010
indeno[123cd]pyrene	4	0.012
dibenz[ah]anthracene	<2	0.001
benzo[ghi]perylene	9	0.015

[1] Blanks = Extraction solvent processed as water samples.

[2] < = Background or samples with values less than the average noise peak area of 300 or 2 ng/0.1 mL sample.

[3] Standard Deviation of samples were derived from the Standard Deviation of lowest Calibtation Standard (Table XX).

TABLE XXIV A. WATER CONCENTRATIONS FOR FLOW-THROUGH DILUTER EXPOSURES

(Low Concentration [1 ng/L] Water)

PAHs	ng / Sample [1]						
	10 °C Day 0	10 °C Day 21	10 °C Average	18 °C Day 0	18 °C Day 21	18 °C Average	26 °C Day 0
naphthalene	39.8	20.4	30.1	9.5	4.5	7.0	5.2
acenaphthylene	9.1	7.2	8.2	7.3	2.0	4.7	19.9
acenaphthene	10.9	12.3	11.6	5.4	7.6	6.5	22.6
fluorene	67.1	10.7	38.9	5.6	2.0	3.8	5.6
phenanthrene	11.1	12.2	11.6	4.8	5.2	5.0	7.6
anthracene	8.3	8.8	8.6	8.8	5.3	7.1	4.2
fluoranthene	7.3	13.3	10.3	40.3	9.3	24.8	45.9
pyrene	10.3	12.4	11.4	5.4	10.6	8.0	225.9
benz[a]anthracene	9.2	13.6	11.4	10.8	2.0	6.4	8.4
chrysene	7.4	12.4	9.9	8.5	2.0	5.3	23.4
benzo[b]fluoranthene	11.1	14.7	12.9	9.6	2.0	5.8	10.6
benzo[k]fluoranthene	10.9	14.0	12.5	7.9	2.0	5.0	6.8
benzo[a]pyrene	6.8	8.3	7.6	10.7	2.0	6.4	8.1
indeno[123cd]pyrene	8.2	9.0	8.6	3.0	8.6	5.8	73.4
dibenz[ah]anthracene	6.0	8.6	7.3	3.6	2.0	2.8	9.1
benzo[ghi]perylene	10.5	12.4	11.5	3.7	7.0	5.4	14.6

[1] Sample = 8 L of ~1 ng/L Diluter water.

TABLE XXIV B. WATER CONCENTRATIONS FOR FLOW-THROUGH DILUTER EXPOSURES

(Low Concentration [1 ng/L] Water)

PAHs	ng/L			
	10 °C Average	18 °C Average	26 °C Average	Study Mean
naphthalene	°(6.0)	1.4	1.0	1.2
acenaphthylene	1.4	0.8	°(3.5)	1.1
acenaphthene	2.0	1.1	°(3.9)	1.5
fluorene	°(4.5)	0.4	0.7	0.5
phenanthrene	1.5	0.6	1.0	1.0
anthracene	1.5	1.2	0.7	1.1
fluorantheue	1.3	°(3.1)	°(5.8)	1.3
pyrene	1.5	1.0	°(29.6)	1.3
benz[a]anthracene	1.4	0.8	1.1	1.1
chrysene	1.4	0.7	°(3.3)	1.1
benzo[b]fluoranthene	1.8	0.8	1.5	1.4
benzo[k]fluoranthene	2.0	0.8	1.1	1.3
henzo[a]pyrene	1.1	0.9	1.2	1.1
indeno[123cd]pyrene	1.3	0.9	°(11.4)	1.1
dibenz[ah]anthracene	1.2	0.5	1.5	1.1
benzo[ghi]perylene	2.0	1.0	2.6	1.9

°( ) = 3 x nominal interference peak, results not used in calculations.

TABLE XXIV C. WATER CONCENTRATIONS FOR FLOW-THROUGH DILUTER EXPOSURES
(Medium Concentration [10 ng/L] Water)

PAHs	ng / Sample [1]								
	10 °C Day 0	10 °C Day 21	10 °C Average	18 °C Day 0	18 °C Day 21	18 °C Average	26 °C Day 0	26 °C Day 21	26 °C Average
naphthalene	32.9	35.7	34.3	35.5	25.9	30.7	24.6	18.3	21.5
acenaphthylene	29.8	32.3	31.1	28.6	21.9	25.3	27.4	9.6	18.5
acenaphthene	28.3	36.0	32.2	71.8	13.1	42.4	33.9	16.2	25.1
fluorene	42.0	46.5	44.3	22.2	18.7	20.5	50.3	16.2	33.3
phenanthrene	42.0	35.9	39.0	24.3	22.1	23.2	37.7	23.2	30.5
anthracene	33.2	37.2	35.2	36.8	19.1	28.0	29.1	16.2	22.7
fluoranthene	38.0	38.8	38.4	33.9	54.8	44.4	66.7	28.8	47.8
pyrene	37.8	39.0	38.4	16.5	23.1	19.8	35.1	24.4	29.8
benz[a]anthracene	42.5	42.1	42.3	54.5	39.5	47.0	45.6	23.3	34.5
chrysene	41.0	42.5	41.8	40.8	34.7	37.8	47.7	32.7	40.2
benzo[b]fluoranthene	40.9	41.7	41.3	52.6	45.9	49.3	46.7	30.8	38.8
benzo[k]fluoranthene	38.3	41.5	39.9	43.8	37.3	40.6	34.0	30.7	32.4
benzo[a]pyrene	35.3	46.5	40.9	49.1	43.9	46.5	37.1	28.9	33.0
indeno[123cd]pyrene	33.3	33.4	33.3	40.5	38.2	39.4	32.0	25.8	28.9
dibenz[ah]anthracene	30.4	30.6	30.5	43.1	36.2	39.7	30.5	22.7	26.6
benzo[ghi]perylene	36.6	37.2	36.9	46.7	48.2	47.5	46.0	30.7	38.4

[1] Sample = 4 L of ~10 ng/L Diluter water.

TABLE XXIV D. WATER CONCENTRATIONS FOR FLOW-THROUGH DILUTER EXPOSURES

(Medium Concentration [10 ng/L] Water)

PAHs	ng/L			
	10 °C Average	18 °C Average	26 °C Average	Study Mean
naphthalene	13.8	12.3	8.6	11.6
acenaphthylene	10.8	8.8	6.4	8.7
acenaphthene	11.0	14.5	8.6	11.4
fluorene	10.3	4.8	7.8	7.6
phenanthrene	9.7	5.8	7.6	7.7
anthracene	11.9	9.5	7.7	9.7
fluoranthene	9.6	11.1	12.0	10.9
pyrene	10.1	5.2	7.8	7.7
benz[a]anthracene	10.6	11.8	8.7	10.4
chrysene	11.9	10.8	11.5	11.4
benzo[b]fluoranthene	11.7	13.9	11.0	12.2
benzo[k]fluoranthene	13.0	13.2	10.6	12.3
benzo[a]pyrene	11.8	13.4	9.5	11.6
indeno[123cd]pyrene	10.4	12.2	9.0	10.5
dibenz[ah]anthracene	10.3	13.4	9.0	10.9
benzo[ghi]perylene	13.2	16.9	13.7	14.6

TABLE XXIV E. WATER CONCENTRATIONS FOR FLOW-THROUGH DILUTER EXPOSURES
(High Concentration [100 ng/L] Water)

PAHs	ng / Sample [1]												
	10 °C Day 0	Rep 1 10 °C Day 21	Rep 2 10 °C Day 21	10 °C Mean	18 °C Day 0	Rep 1 18 °C Day 21	Rep 2 18 °C Day 21	18 °C Mean	Rep 1 26 °C Day 0	Rep 2 26 °C Day 0	Rep 1 26 °C Day 21	Rep 2 26 °C Day 21	26 °C Mean
naphthalene	103.2	114.3	116.9	111.5	114.3	80.5	82.2	92.3	101.1	99.0	75.4	83.7	89.8
acenaphthylene	138.1	159.7	161.7	153.2	175.7	101.6	129.1	135.5	203.4	174.0	112.7	126.8	154.2
acenaphthene	138.9	163.2	165.2	155.8	155.7	132.6	136.5	141.6	183.5	209.0	108.7	117.3	154.6
fluorene	155.4	168.8	173.7	166.0	304.0	133.9	138.0	192.0	309.7	282.4	122.4	146.6	215.3
phenanthrene	159.6	179.3	182.6	173.8	184.8	135.1	156.5	162.6	260.1	260.9	137.4	149.2	201.9
anthracene	144.9	173.5	178.2	165.5	157.5	105.3	128.9	130.6	273.2	277.9	128.4	123.9	200.9
fluoranthene	158.5	173.8	174.4	168.9	209.7	176.3	169.0	185.0	246.0	280.5	150.0	164.6	210.3
pyrene	154.7	166.8	168.4	163.3	188.4	139.2	155.7	161.1	256.2	243.6	151.6	142.2	198.4
benz[a]anthracene	163.7	175.1	175.2	171.3	242.6	188.0	226.4	219.0	301.9	298.0	153.2	161.2	228.6
chrysene	161.7	173.2	171.6	168.8	216.4	161.9	185.2	187.8	225.6	239.7	136.0	146.6	187.0
benzo[b]fluoranthene	166.6	163.2	168.1	166.0	235.0	195.2	308.5	246.2	312.6	255.3	192.9	194.8	238.9
benzo[k]fluoranthene	146.7	166.7	158.5	157.3	195.6	167.8	249.6	204.3	181.3	222.7	162.9	162.3	182.3
benzo[a]pyrene	145.5	157.1	158.4	153.7	186.3	159.0	265.7	203.7	105.3	203.6	163.3	158.4	157.7
indeno[123cd]pyrene	115.1	131.8	133.7	126.9	191.4	142.1	293.8	188.5	199.8	2.0	167.9	171.7	135.4
dibenz[ah]anthracene	100.0	115.0	120.3	111.8	188.0	132.5	279.2	199.9	183.2	167.7	167.7	154.6	168.3
benzo[ghi]perylene	103.4	134.1	140.7	126.1	181.5	162.8	291.9	212.1	190.0	205.3	198.4	177.9	192.9

[1] Sample = 2 L of ~100 ng/L Diluter water.

TABLE XXIV F. WATER CONCENTRATIONS FOR FLOW-THROUGH DILUTER EXPOSURES
(High Concentration [100 ng/L] Water)

PAHs	ng/L			
	10 °C Mean	18 °C Mean	26 °C Mean	Study Mean
naphthalene	89.6	74.2	72.2	78.7
acenaphthylene	106.7	94.3	107.4	102.8
acenaphthene	106.5	96.9	105.8	103.1
fluorene	77.5	89.6	100.5	89.2
phenanthrene	86.9	81.3	101.0	89.7
anthracene	112.3	88.6	136.3	112.4
fluoranthene	84.8	92.9	105.6	94.4
pyrene	85.5	84.3	103.9	84.9
benz[a]anthracene	86.1	110.1	114.9	103.7
chrysene	96.4	107.2	106.7	103.4
benzo[b]fluoranthene	94.0	139.4	135.3	122.9
benzo[k]fluoranthene	102.7	133.4	119.0	118.3
benzo[a]pyrene	88.6	117.5	90.9	99.0
indeno[123cd]pyrene	78.9	117.3	84.2	93.4
dibenz[ah]anthracene	75.7	135.4	114.0	108.4
benzo[ghi]perylene	90.0	151.5	137.8	126.4