Laboratory Comparisons of Oysters and Semipermeable Membrane Devices (SPMDs)

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Chapter 1
INTRODUCTION

Sir Percival Pott, the British surgeon, reported in 1775 the high frequency of cancer appearing in chimney sweeps (Pott 1775), however more than 150 years elapsed before the carcinogenic agents in pitch were identified as polyaromatic hydrocarbons (PAHs). Two hundred years after Dr. Pott, PAHs and their derivatives have been found to be the largest single chemical class of carcinogens (Bjørseth 1983). Because of the potential risk posed by PAHs, standards for drinking water, food, effluents and other sources have been set by many agencies. However, the measurement of PAHs in various media, particularly water, has remained problematic.

The Minamata disaster (Taylor 1986) and similar events connected water quality to human health and human activities. In 1975 Goldberg suggested in his article, "The Mussel Watch - A first step in global marine monitoring", that mussels would be a cost effective method for surveying the coastal environment for trace contaminants and coined the term "sentinel organism" (Goldberg 1975). In his monograph, Phillips outlined the necessary criteria for selecting a "biomonitor", (Phillips 1980). From this viewpoint, Table 1-1, bivalves possess several attractive features; their widespread natural occurrence, high tolerance and viability, relative ease of analysis, and high bioconcentration factors for a wide range of compounds. However, the effects of stress, interspecies variability, uncertainties about uptake, depuration and biotransformation of contaminants convolute data and make quantitative statements about water concentrations difficult. In fact, despite decades of study and use both nationally and internationally, water criteria are not based on any biomonitoring data.

Based on Phillips' criteria given in Table 1-1 the use of SPMDs as surrogate biomonitors provides several important advantages.

Table 1-1 Characteristics of Sentinel Organisms and Contaminant Information

Characteristic	Contaminant Information
Sedentary	Spatial information about contaminant concentrations
Abundant	Many samplings of an area or range of areas
Long Lived	Temporal trends about contaminant concentrations
Sufficient Size	Large enough to produce sufficient signal
Easy to handle	In field and laboratory work
Tolerant of conditions (hardy)	Wide range of interesting areas may be surveyed
"Accumulator"	Must accumulate compounds of interest without being adversely effected
Organism concentrations related to environmental concentrations	"All organisms of a given species used should exhibit the same correlation between their pollutant content and the average pollutant content in the surrounding water at all locations studied, under all conditions." (Phillips 1980)

Spatial Trends: "Sedentary"

Transplanted bivalves have the same kind of mooring concerns as SPMDs if spatial trends are to be discerned. SPMDs tend to require more complicated fixtures than the nylon mesh bags used for bivalves and are more similar to the situation for caged fish. Analytical and sampling variance will determine the level of spatial discrimination possible.

Sampling Frequency: "Abundance"

The high abundance of bivalves, natural or cultivated, throughout the world has made them the most successful form of biomonitor in coastal areas. (In the same way, fish have been successful for most inland waterways). However the difficulty for bivalves (and fish) is that there seldom is a single species available for spatial surveys over vast or sometimes even moderate geographical areas (Goldberg, Bowen et al. 1978). A case in point is the International Mussel Watch survey of

the Americas where 27 different species of bivalves were required to complete the survey (Sericano, Wade et al. 1995).

Sampling Duration: "Long Lived"

Typically, exposure durations are much shorter than biomonitoring organism lifetimes so the criteria of longevity is easily met. For monitoring with transplanted organisms, achieving steady state with respect to the accumulated contaminants is often the criteria used to determine exposure durations although food and water quality may create practical limitations. SPMDs have only mechanical and in some cases fouling constraints on their "lifetimes". Under extreme conditions wear of the polyethylene is inevitable however deployment durations are usually on the order of a month and membrane fatigue is therefore infrequent. Properly protected SPMD "lifetimes" are generally sufficient to provide data on contaminants over "interesting periods" of time (experience has shown that 90 day deployments are quite feasible).

Application: "Ease of handling" & "Size"

Bivalves can be used by either transplanting foreign organisms or collecting indigenous organisms. Transplant experiments bear the most similarity to SPMD deployments. Placing 30 to 50 bivalves in a nylon mesh bag is simpler than SPMD deployments which do require special fixtures and preparation. However the ease of handling the sampling matrices in the laboratory is an important concern. There is a considerable time investment in the process of preparing bivalves; shucking, homogenizing and extracting. The necessary parameters of wet, dry and lipid weights present issues of analytical concern. For example, the class of lipids extracted is highly solvent and method dependent (e.g., (Kates 1986)). From an analytical (signal to noise) viewpoint, one SPMD is equivalent to roughly 5 to 10 grams of bivalves tissue depending on the particular analyte and the exposure periods. In terms of the total interferences that must be eliminated, mussels are approximately 1%-2% lipid, oysters 2%-4% (fish range widely from .03% to about 10%), so typically the lipid for bivalves is greater than 100 milligrams per set, while each SPMD has less than approximately 50 milligrams of total extractables. SPMDs are a much simpler matrix with fewer interferences both gravimetrically and chemically. Overall the

total time invested in extracting analytes from SPMDs is larger (due to the unattended dialytic step) however the active or "hands on" time invested by laboratory staff is roughly 15% of that required for bivalves.

Other Analytical and Methodological Concerns - QA/QC

SPMD extracts are readily amenable to *in vitro* bioassay techniques such as Microtox®, Mutatox® (Microbics Corporation, Carlsbad, CA) and EROD allowing the estimation of the toxicological significance of bioavailable contaminants to be studied (Huckins, Petty et al. 1996).

Transplant experiments require "clean" control organisms which are frequently difficult to obtain. Pristine SPMDs can be manufactured in a very reproducible manner with a well defined history of handling. Standard Reference Materials (SRMs) for tissues and other matrices are very difficult to manufacture, certify, handle and store (e.g., (Wise, Benner et al. 1991)). Repeated freezing and thawing alters wet weights; one of several reasons that necessitates reporting bivalve SRM results on a dry weight basis. It is anticipated that a standard SPMD SRM would be much simpler to create, store and use.

Range: "Tolerance of Environmental Conditions"

Bivalve viability is restricted by salinity, turbidity, temperature, dissolved oxygen and other water quality issues. Bivalve filtration and ventilation rates are also influenced by these variables (Bayne 1976). Because oysters have a wider range of salinity tolerance than mussels, e.g., *Mytilus californianus* tolerates salinities from 17 parts per thousand to seawater (Bayne 1976), whereas oysters tolerate 25 parts per thousand to 0.5 parts per thousand salinity, oysters have been more successfully applied in monitoring estuarine environments. Because SPMDs are inanimate, there are no problems involving these environmental factors. In general, SPMDs are applicable in any aquatic environment and many interesting and important areas which can not be surveyed by biomonitors, can be studied with SPMDs. Biofouling and temperature are the only environmental variables that significantly effect SPMD performance. However, SPMDs have

been shown to be effective samplers in water at or below 0°C and in waters with high biological activity.

Chemical Range: "Accumulators"

The vast majority of the organochlorine compounds routinely measured in bivalves and all the priority pollutant PAHs have been detected in SPMDs. Low levels are expected for the more chlorinated phenols which are only sequestered by SPMDs in their unionized form which is some fraction of the total chlorinated phenol (Herve, Prest et al. 1995). Exceptionally polar compounds, those which have octanol-water partitioning coefficients in the hundreds or lower and high aqueous solubilities, are expected to have low propensities for accumulating in SPMDs both because of the non-polar membrane and interior the lipid phase.

Proportionality to Environmental Concentrations

Phillips' description of this qualification for biomonitors has yet to be developed to the point of standardizing water quality criteria on the basis of organism derived concentrations. A wide range of biological variables effect contaminant levels in organisms such as biochemical factors, seasonal lipid variations (Capuzzo, Farrington et al. 1989), gender, age, etc., as well as environmental conditions like turbidity, dissolved oxygen, temperature, etc., and so wide variations can be found for bioconcentration and bioaccumulation factors. Huckins et al. have developed and demonstrated a clear correspondence between water concentrations and SPMD sequestering (Huckins, Manuweera et al. 1993). In application, the device produces accurate and highly reproducible measurements (Ellis, Huckins et al. 1995).

HYDROCARBONS AND PAHS IN BIVALVES

In his original article, Goldberg suggests using mussels as "sentinels for hydrocarbon pollution". This in spite of the fact he also cites that "*Mytilus edulis* rapidly responds to the total hydrocarbon burden of its environment through uptake in its tissues and also rapidly releases such pollutants upon exposure to clean waters". This "rapid response" represents a serious disadvantage in not providing a time-integrated picture of contaminant exposure if environmental

concentrations vary during the exposure. However, this intimated (hydrocarbon) contaminant dynamics in bivalves needed to be well characterized if biomonitoring information was to provide insight into exposure. Many studies have pursued the measurements of bioconcentration (or bioaccumulation) factors but fewer have sought to determine the kinetic parameters of uptake and clearance constants.

Modeling PAH accumulation in Oysters

In the first approximation, single compartment, reversible kinetics have been successfully applied to oysters and other bivalves since uptake across their biomembranes is considered passive and metabolism is low. Essentially this assumes the organism is a single homogeneous compartment passively sequestering the contaminant from the surrounding water. Because of the hydrophobic nature of the contaminants, the mollusk compartment of concern is considered to be just the lipid (Smith, Witkowski et al. 1988) which are primarily triacylglycerides. This assumption has been relatively successful since lipid based bioconcentration factors, L-BCFs, correlate fairly well with octanol - water partition coefficients, K_{ow}s , and with the inverse of the water solubilities (e.g, (Chiou, Freed et al. 1977)). Based on this point of view, all bivalves are the same; thus differences in species are considered secondary. (Recent measurements of species specific metabolic capabilities and their possible influence on contaminant burdens have called this approximation into question (e.g., (Solé, Porte et al. 1994).) The apparent early success of lipid - water partitioning models (to within an order of magnitude) and the observed independence of bivalve BCFs for hydrocarbons over a wide range of exposure concentrations were arguments for "using bivalves as sentinel organisms for monitoring petroleum pollution in coastal waters" (Burns and Smith 1981).

The majority of bioaccumulation and kinetic studies have focused on hydrocarbon accumulation in mussels, instead of oysters, and illustrate the difficulties in determining BCFs (e.g., (Pruell, Lake et al. 1986), (Murray, Richardson et al. 1991)). Issues of representative and accurate dissolved phase water concentrations, the role of particulates, verification of steady state, stress, etc., complicate data and compromise conclusive interpretation.

An early laboratory study exposed oysters (*Crassotrea virginia*) to petroleum hydrocarbons in filtered (1 μm) seawater (Stegeman and Teal 1973). The rate of hydrocarbon accumulation measured after 2 days of exposure correlated directly with the aqueous hydrocarbon concentration for exposures from 100 to 450 μg/L; whereas at 900 μg/L the rate was suppressed. Stegeman and Teal also found a higher percentage of aromatics than saturated hydrocarbons present in the tissues than in the oil, implying a greater concentration factor for these components. In depuration, 90% of the hydrocarbons were lost in 2 weeks. A "leveling off" or slow discharge was observed for the remaining 10% over a month. This biphasic depuration, and the observation that the initial rate (2 days) exhibited a dependence on wet weight while the longer exposures switched to a lipid basis, were used as support for a multi-(2)-compartment model; a rapidly saturated compartment and another more kinetically isolated or slow compartment. However, steady state was not reached even after 49 days of exposure so no BCFs was determined (a lipid-based concentration factor at day 49 was approximately 2×10^5). Analytical and experimental problems may have influenced the data; e.g., hydrocarbons were measured (at 11 μg/L) in the "clean" water used for depuration.

Another (*Crassotrea virginia*) oyster study by Bender et al., modeled PAH bioconcentration factors parameters in laboratory exposures of oysters to contaminated sediments (Bender, Hargis et al. 1988). Three oysters were sampled at 3, 7, 14, 21 and 28 day intervals during the 28 day exposure and 28 day depuration phase. Water was sampled weekly. Model parameters were obtained by computer fitting to the data and equilibrium bioconcentration factors, BCF_es, and uptake and clearance rates calculated, Table 1-2. No units are cited[1] but it can be inferred that (perhaps) the BCF_e is on a dry weight basis, k_2 is in day^{-1}, and k_1 may be milliliters (or grams) of water day^{-1} gram dry $weight^{-1}$. No data or uncertainties are cited and the significant figures in reported values are similarly unjustified. The data suggests PAH half-lives on the order of two weeks and uptake on the order of a liter per day per gram dry weight. The data trends are consistent with what is physically expected; decreasing k_2 values and increasing BCFs with increasing log K_{ow}s. It is interesting that the benzo(g,h,i)perylene and chrysene BCFs are

[1] Professor Bender was unavailable for clarification since he has been appointed by President Clinton to a directorship of R&D at the EPA.

comparable despite the large difference in their log K_{ow}s, 5.9 and 7.1 respectively. However a number of results are suspect: 1) the benzofluorene isomers have dramatically different k_1 and BCF values even though their K_{ow}s are expected to be similar, 2) phenanthrene has a half-life of only 3.4 days, and 3) benzo(a)anthracene and chrysene have similar k_1, k_2 and BCF values yet physicochemically similar benzopyrene isomers have k_2 and consequently BCF values that differ greatly.

Table 1-2 Uptake, clearance and bioconcentration factors in oysters (*Crassotrea virginia*) at 25°C, (Bender, Hargis et al. 1988)

Compound	k_1	k_2	BCF_e
Benzo(a)anthracene	1310	0.045	28846
Benzo(a)fluorene	508	0.066	8796
Benzo(b)fluorene	1410	0.072	19666
Benzo(a)pyrene	639	0.032	19673
Benzo(e)pyrene	790	0.023	33731
Benzo(g,h,i)fluoranthene	1465	0.056	26206
Benzofluoranthene	794	0.009	84217
Chrysene	1187	0.046	26019
Fluoranthene	821	0.118	6965
Methylphenanthrene	529	0.103	5151
Methylpyrene	2365	0.066	36074
Perylene	817	0.075	10861
Phenanthrene	330	0.206	1604
Pyrene	921	0.104	8857
Total PAH	798	0.053	15190

A recent study by Sericano et al., compared oyster (*Crassotrea virginia*) depuration of PAHs for oysters native to uncontaminated and chronically contaminated areas (Sericano, Wade et al. 1996). Sericano et al. concluded that significant differences existed between biological half-lifes (k_2s) of PAHs in oysters depending on prior exposure. Oysters from more contaminated sites depurated PAHs more slowly than those from the uncontaminated reference site. Half-lifes were reported for 7 non-alkylated, mid to high molecular weight PAHs from phenanthrene to indeno[1,2,3-c,d]pyrene and varied from 9 to 32 days. PAH half-lifes were comparable to those found in mussels (*Mytilus edulis*) by Pruell (Pruell, Lake et al. 1986). Converting the half-lifes to

k_2 values for comparison to the results of Bender et al., reveals fairly good agreement for several PAHs, e.g, benzo(a)anthracene and chrysene, but large differences for others, e.g., fluoranthene and pyrene. No uncertainties were reported so the significance of the disagreements or confidence in the values can be not estimated. No information on uptake rates was reported.

Table 1-3 Calculated k_2 values ($days^{-1}$) for reported PAHs from oysters and mussels (Sericano, Wade et al. 1996)

Compound	Oyster	Oyster	Mussel
Fluoranthene	0.027	0.022	0.023
Pyrene	0.069	0.058	
Benzo(a)anthracene	0.053	0.046	0.039
Chrysene	0.058	0.043	0.050
Benzo(e)pyrene	0.058	0.043	0.050
Benzo(a)pyrene	0.077	0.069	0.046
Indeno(1,2,3-c,d)pyrene	0.069	0.063	0.043

In general, all previous studies suffer from the aforementioned issues of uncertain (dissolved phase) water concentrations, the role of particulates (since most used contaminated sediment as the exposure media), verification of steady state, incomplete statistics and data analysis and frequently analysis of a very limited range of PAHs. If a more concrete understanding of contaminant sequestration by bivalves and their usefulness and application as sentinel organisms is to be obtained, these issues need to be addressed.

STUDY OBJECTIVES

Potential Correlation Between Bivalves And SPMDs

Two possible approaches for comparing bivalve and SPMD accumulation of contaminants are steady state and kinetic. The steady state situation compares their relative capacities for contaminant concentration at pseudo-equilibrium. Chiou has shown a linear correspondence

between the log of triolein - water partition coefficient, K_{tw}, and log K_{ow} for many compounds up to a log K_{ow} of about 5 (Chiou 1985). Consequently, a similar relationship exists between log BCF and log K_{tw};

$$\log BCF = 0.957 \log K_{tw} + 0.245$$

Since triacylglycerides are a major component of bivalve lipid and triolein is the sequestering phase in SPMDs, the relation suggests a linear (and nearly one to one) correspondence is possible for PAH lipid normalized concentration factors in bivalve and SPMDs (if bivalve metabolism is not significant).

Structurally there is an analogy between bivalves and SPMDs; a surface (gill) interposed between a sequestration media and the environment. Mussel gill areas are about 0.03 to 0.06 cm^2 gram dry $weight^{-1}$ and filtration or ventilation rates are roughly ½ to 1½ liters h^{-1} gram dry $weight^{-1}$; thus bivalve pumping exchanges a tremendous amount of water in a day and keeps them well perfused. The standard one HUCK SPMD has roughly 400 cm^2 , much more than a bivalve, but relies on passive exchange with the surrounding water.

Study Objectives

The intention of this work is to compare SPMDs and oysters with respect to their analyte sequestering capacities and (dissolved phase) kinetics for a series of interesting and important PAH compounds. The objectives are to measure the relevant kinetic parameters and to use this information to compare and contrast their abilities as environmental monitors and evaluate the degree of correspondence in their behavior. Potentially this information may be useful in understanding the large database presently available from bivalve monitoring programs and aid in future program design. It has been suggested that SPMDs may represent suitable surrogates for aquatic organisms and may provide insight into their steady state and kinetic behavior (Huckins, Tubergen et al. 1990). The comparison of SPMDs and oysters will evaluate the potential "scaling" of this synthetic surrogate to the widely accepted biomonitoring organisms.

Study design

Briefly, oysters and SPMDs were exposed to filtered seawater containing 10 ppt, 100 ppt and 250 ppt (nominal) concentrations of the selected PAHs. After 5, 10, 15 and 20 days of exposure the samples were analyzed for accumulated PAHs. A more detailed description of the experimental design is presented in the next chapter.

Study Compounds

Previous laboratory studies of SPMD sequestration kinetics focused on the 16 priority pollutant PAHs. For this study the list of compounds was expanded to include other PAHs, alkylated PAHs and three heterocyclic AHs, Table 1-4. The additional compounds, especially the alkylated isomers, are expected to provide further insight into the effect of changes in compound structure on SPMD and oyster uptake. Understanding the SPMD kinetics for these components will be important for characterizing petroleum in the environment as in alkyl-phenanthrene / alkyl-dibenzothiphene double ratio plots (Brown and Boehm 1993). (Two of the compounds are particularly interesting for toxicological reasons, i.e., benzo[a]pyrene and 3-methylcholanthrene are considered potent carcinogens, e.g., (Cooke and Dennis 1983)).

Table 1-4 lists the names of the compounds studied and an index number. This index number is (infrequently) used to refer to the compound identity in certain plots elsewhere in the report. Compounds names are given as both the common name and IUPAC synonym. Figure 1-1 and Figure 1-2 show the PAH carbon backbone structures in the Kekulé rendering.

Polyaromatic Hydrocarbons Nomenclature

Throughout this manuscript the term PAH will be used for polycyclic-heteronuclear aromatic hydrocarbon or just polyaromatic hydrocarbon to indicate the aromaticity and predominately hydrocarbon nature of the compounds studied.

Table 1-4 PAH Compound List and Figure Index

Index	Compound names
1	naphthalene
2	benzo[b]thiophene
3	2-methylnaphthalene
4	1-methylnaphthalene
5	biphenyl
6	1-ethylnaphthalene
7	1,3-dimethylnaphthalene
8	acenaphthylene
9	acenaphthene (1,8-hydroacenaphthylene)
10	4-methylbiphenyl
11	2,3,5-trimethylnaphthalene
12	fluorene (diphenylenemethane)
13	1-methylfluorene
14	dibenzothiophene
15	phenanthrene
16	anthracene
17	2-methylphenanthrene
18	9-methylanthracene
19	3,6-dimethylphenanthrene
20	fluoranthene (1,2-benzacenaphthene)
21	pyrene
22	2-methylfluoranthene
23	benzo[b]naphtho[2,1-d]thiophene
24	benz[a]anthracene (1,2-benzanthracene)
25	chrysene
26	benzo[b]fluoranthene (2,3-benzofluoranthene)
27	benzo[k]fluoranthene (8,9-benzofluoranthene)
28	benzo[e]pyrene (4,5-benzopyrene)
29	benzo[a]pyrene
30	perylene
31	3-methylcholanthrene
32	indeno[1,2,3-c,d]pyrene
33	dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)
34	benzo[g,h,i]perylene (1,12-benzoperylene)

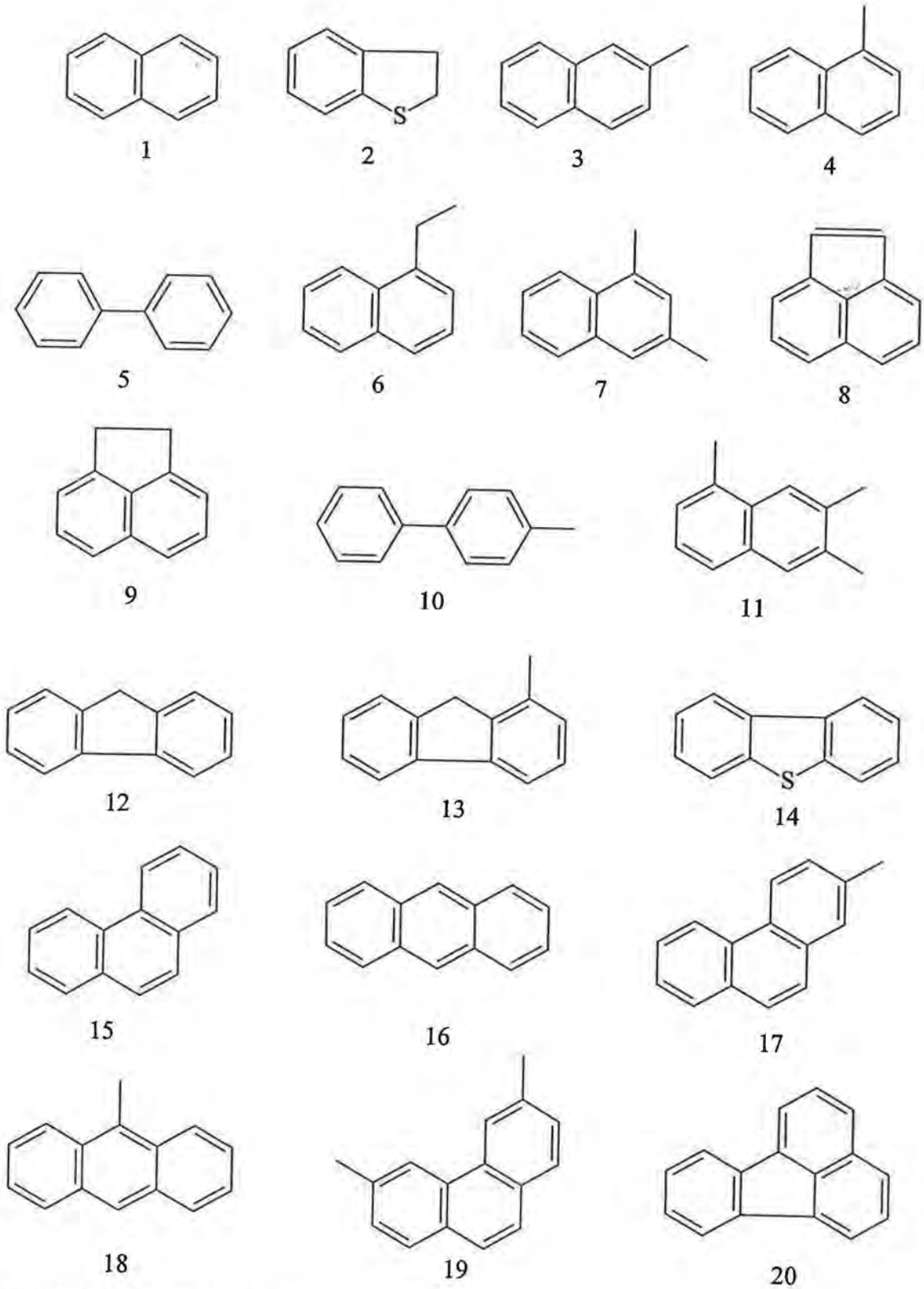

Figure 1-1 PAH Analytes

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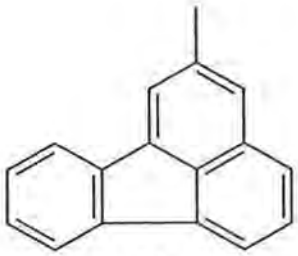

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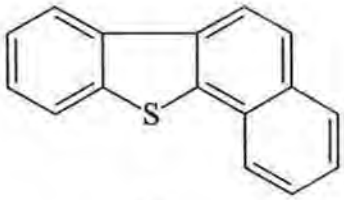

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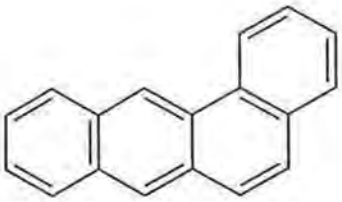

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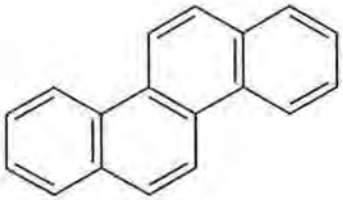

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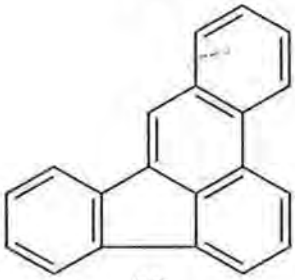

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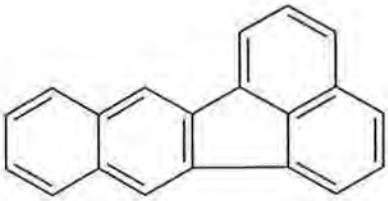

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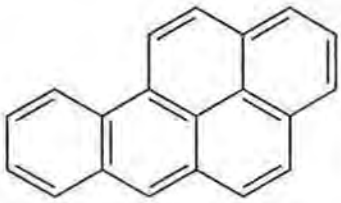

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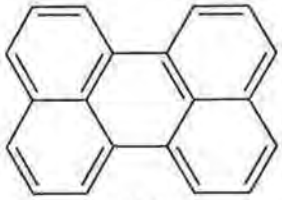

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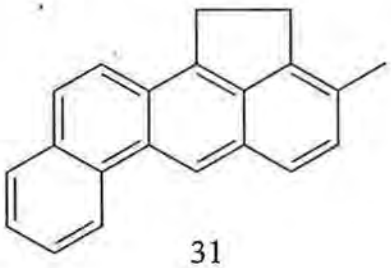

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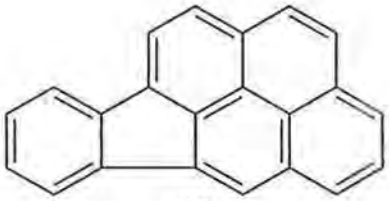

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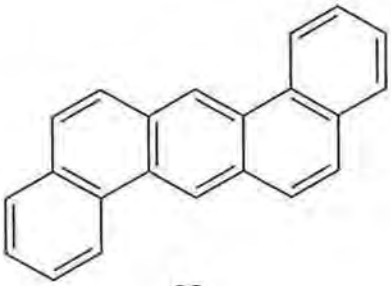

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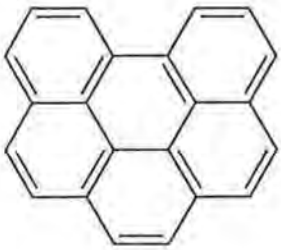

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Figure 1-2 PAH Analytes

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Chapter 2

EXPERIMENTAL OVERVIEW

In this section an overview of the experimental design is provided with a summary of the significant steps. More detailed descriptions of the procedures, methods, and any pertinent data files are provided in the various appendices.

The four major sections of the study are the Diluter Exposure, Sample Processing, Sample Analysis, and the Results, Figure 2-1.

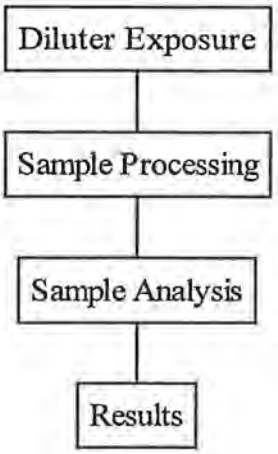

Figure 2-1 Study and Report Overview

DILUTER EXPOSURE

Bivalve Preparation

For each study, 50 "medium sized" oysters (*Crassostrea gigas* - Mayagi) were incubated in laboratory seawater for (not less) (at least) than 2 weeks. Barnacles and other encrustations were removed from the shell before incubation. Oysters were fed a mixture of algae every other day throughout incubation. A set of oysters were set aside as controls at the end of an incubation period or flow-through trial.

SPMD Preparation

SPMDs were prepared from 3 mil thick, 2.5 cm wide polyethylene layflat tubing by researchers at the MSC (according to their protocols). The SPMDs were approximately 45.7 cm (18 inches) long which is half the length of the standard, one HUCK SPMD. Lipid in each SPMD was spiked with perdeuterated phenanthrene to serve as a permeation reference compound (PRC). SPMDs arrived at LML sealed in solvent rinsed tin cans. Each trial had SPMD laboratory blanks (which remained at MSC), trip blanks, diluter (seawater) controls, and exposed SPMDs.

Diluter Preparation & Design

The diluter system consists of a filter system, two pumping systems, a delivery or mixing manifold and the exposure chambers. The filter system is composed of a series of polypropylene housings, Kynar or polypropylene connections and high capacity filters to provide clean seawater filtered down to 0.1 micron (nominal). Seawater is pumped into the chambers by the main pump which is calibrated to deliver seawater at 400 mL/min (±1%) prior to each experiment. Similarly the spiking pump is calibrated to deliver 0.004 mL/min (±2%) of methanol into the mixing manifold. Spiked seawater from the mixing manifold is distributed to each of the chambers by a delivery manifold constructed of glass, Teflon tubing and Kynar connectors. Teflon needle valves on the delivery manifold regulate flow to each of the chambers. Flows from the chambers were measured at the chamber outlets and varied no more than about ±15% over the course of an experiment. Flow variation was caused by biofouling of chambers and tubing as described below. Water temperature was measured at the chamber outlets to ±0.05 °C by mercury thermometer. The 3 liter, cylindrical exposure chambers were entirely of glass except for a silicone o-ring seal.

Two important features of the diluter design are (1) the completely enclosed system allows mass balances to be considered, and (2) delivered concentrations and temperatures are identical for all chambers. In other words, both bivalves and SPMDs received the same profile of PAHs under the same conditions.

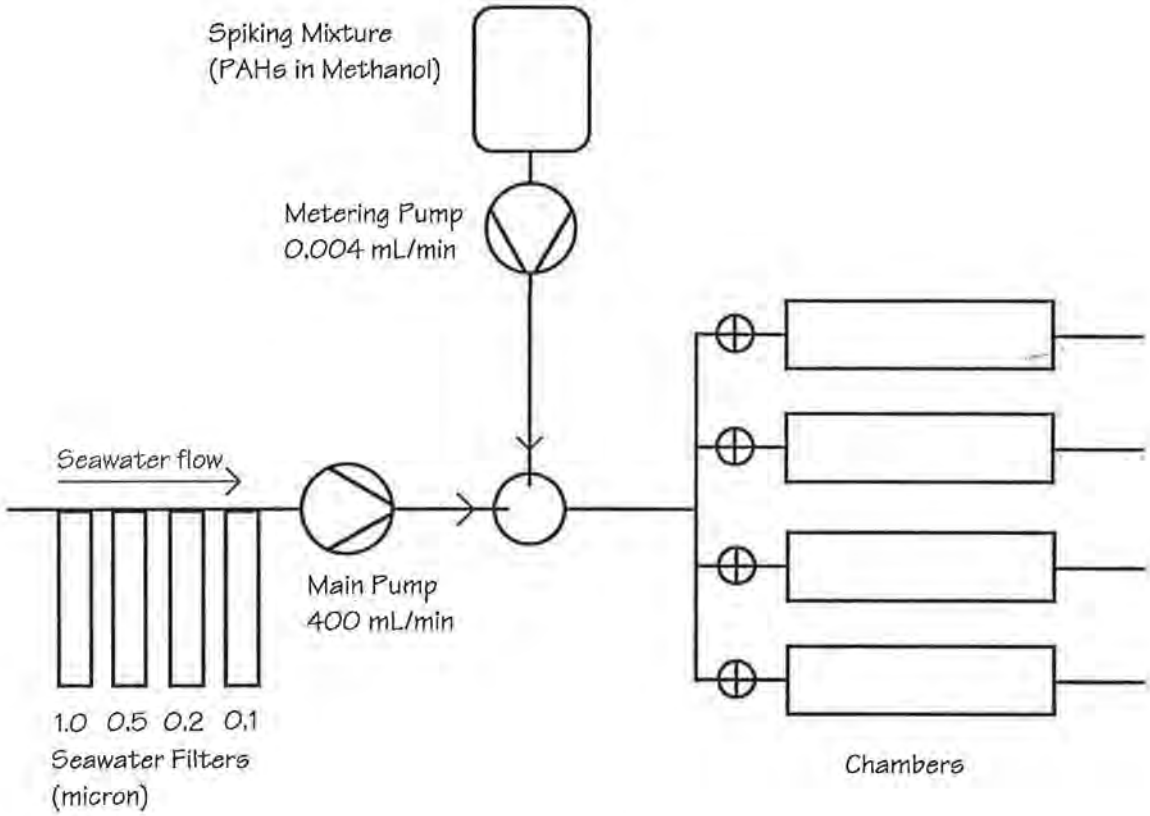

Figure 2-2 Schematic of Diluter Arrangement

Spiking solutions were made in methanol from a master provided by the MSC at a concentration 10^4 higher than that desired for the trial; i.e. the spiking solution would be diluted 1 / 10,000 at delivery to produce the 10 ppt, 100 ppt, and 250 ppt exposure concentrations. The spiking solution was delivered for at least 6 hours prior to sample exposures to purge the system and saturate surfaces.

Chamber & Sampling Schedule

Every 5 days, oysters and SPMDs were removed from the chambers, and water samples and SPMD controls were taken. Table 2-1 shows the chamber schedule and number of replicates for each exposure duration. There were always 6 SPMDs or 6 oysters in each chamber to produce a constant demand. Two chambers were devoted to bivalves and two for SPMDs. New chambers were used every 5 days to prevent or minimize the adverse effects of biofouling. Biofouling

originated in chambers containing bivalves and would spread throughout the system if unchecked.

Table 2-1 Chamber #1 is indicated by C1 and Chamber #2 by C2. The notation indicates Chamber Number-*Type*-days of exposure- replicate. Either SPMD or Oyster can be the sample type.

Position	0 to 5 days	5 to 10 days	10 to 15 days	15 to 20 days
1	C1-*sample*-20-A	C1-*sample*-20-A	C1-*sample*-20-A	C1-*sample*-20-A
2	C1-*sample*-20-B	C1-*sample*-20-B	C1-*sample*-20-B	C1-*sample*-20-B
3	C1-*sample*-20-C	C1-*sample*-20-C	C1-*sample*-20-C	C1-*sample*-20-C
4	C1-*sample*-20-D	C1-*sample*-20-D	C1-*sample*-20-D	C1-*sample*-20-D
5	C1-*sample*-20-E	C1-*sample*-20-E	C1-*sample*-20-E	C1-*sample*-20-E
6	C1-*sample*-20-F	C1-*sample*-20-F	C1-*sample*-20-F	C1-*sample*-20-F
Position	0 to 5 days	5 to 10 days	10 to 15 days	15 to 20 days
1	C2-*sample*-5-A	C2-*sample*-15-A	C2-*sample*-15-A	C2-*sample*-15-A
2	C2-*sample*-5-B	C2-*sample*-15-B	C2-*sample*-15-B	C2-*sample*-15-B
3	C2-*sample*-5-C	C2-*sample*-15-C	C2-*sample*-15-C	C2-*sample*-15-C
4	C2-*sample*-5-D	C2-*sample*-15-D	C2-*sample*-15-D	C2-*sample*-15-D
5	C2-*sample*-10-A	C2-*sample*-10-A	C2-*sample*-10-B	C2-*sample*-10-B
6	C2-*sample*-10-C	C2-*sample*-10-C	C2-*sample*-10-D	C2-*sample*-10-D
Exposure Durations	5 days	10 days	15 days	20 days
Replicates	4	4	4	6

SPMDs were mounted onto 316 stainless steel wire frames and arranged to minimize contact. Oysters were not mounted to avoid stress but placed throughout a chamber.

SAMPLE PROCESSING

Three types of samples were processed prior to GC-MS-SIM analysis; SPMD, oyster, and water. A summary of the sample processing is provided in the following sections. More detailed descriptions of the methodologies can be found in the Analytical Methodology Appendix.

SPMDs

SPMDs were processed for PAHs by the MSC. A 50% split of each processed SPMD was received as an ampouled sample with recovery surrogate verification spikes and related QA/QC

samples. These were transferred, condensed and injected under the same conditions as other samples. Sample volumes were adjusted prior to injection to account for the differing exposure concentrations and SIM quantitation range and detection limits.

Oysters

Oysters were shucked with an oyster knife with a clean stainless steel blade. Fluid was drained from inside the shell before the tissue was removed and homogenized. Tissues were characterized by wet, dry and lipid weights. Dry weights were determined on a small subset of the homogenized tissue. Homogenized samples were spiked with deuterated recovery surrogates and the lipid phase extracted with dichloromethane. Determining total extractable lipids required sacrificing about 20% of the extracted sample. The lipid extracts were fractionated for PAHs on an alumina-silica open column. Condensed column eluates were spiked with an HPLC instrumental standard and polished by (high pressure) size exclusion chromatography (SEC). The condensed SEC eluates were spiked with GC-MS PAH-ISTD and analyzed by HRGC-MS in the selected ion monitoring mode.

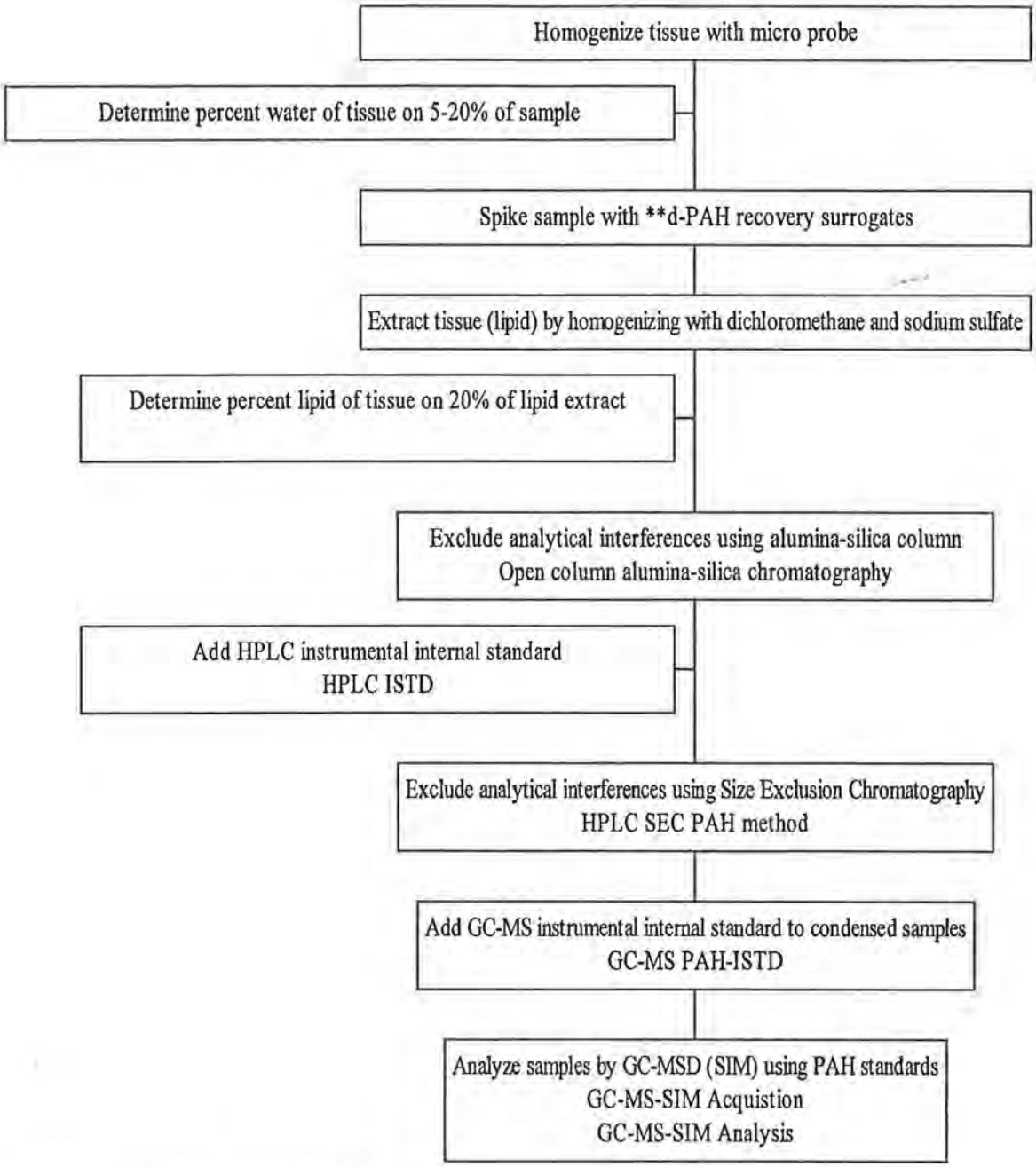

Figure 2-3 Oyster Processing Summary

Water

Water samples were taken directly from the exposure chamber outlets using clean 4L amber bottles and so represents an average over all four chambers during the sampling. Water samples were spiked with deuterated recovery surrogates, agitated (by sediment roller or sonicater) and

extracted in a separatory funnel with dichloromethane. The sample extracts were eluted from an alumina-silica column, condensed, spiked with GC-MS PAH-ISTD and analyzed by GC-MS.

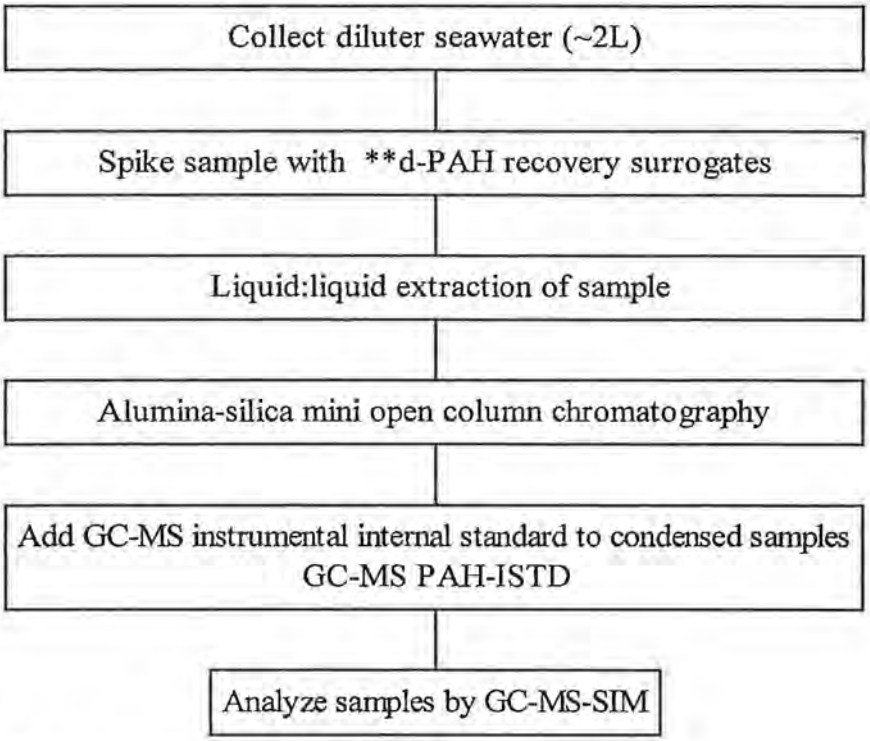

Figure 2-4 Water Sample Processing Summary

SAMPLE ANALYSIS

GC MS Method Overview

Mass spectrometric detection by selected ion monitoring (SIM) requires (1) establishing a gas chromatographic method that provides stable retention times with sufficient separation and (2) identifying ions which have sufficient abundance and fewest potential interferences for the analytes of interest, Figure 2-5. Quantification requires measurements of instrument response (signal) for several known concentrations of the analytes over an extended range; e.g., an order of magnitude. Polyaromatic species have an intense molecular ion (M+), which is commonly the most abundant (base peak), and frequently a peak at one or two mass units lower (M-1+, M-2+)

which serve as confirming ions. Usually there are few other ions that provide enough intensity to be suitable confirming ions and there is an increasing potential for interferences as one looks at lower m/e ions. Since PAH isomers have equivalent molecular weights, not all the ions are unique and retention time information becomes important.

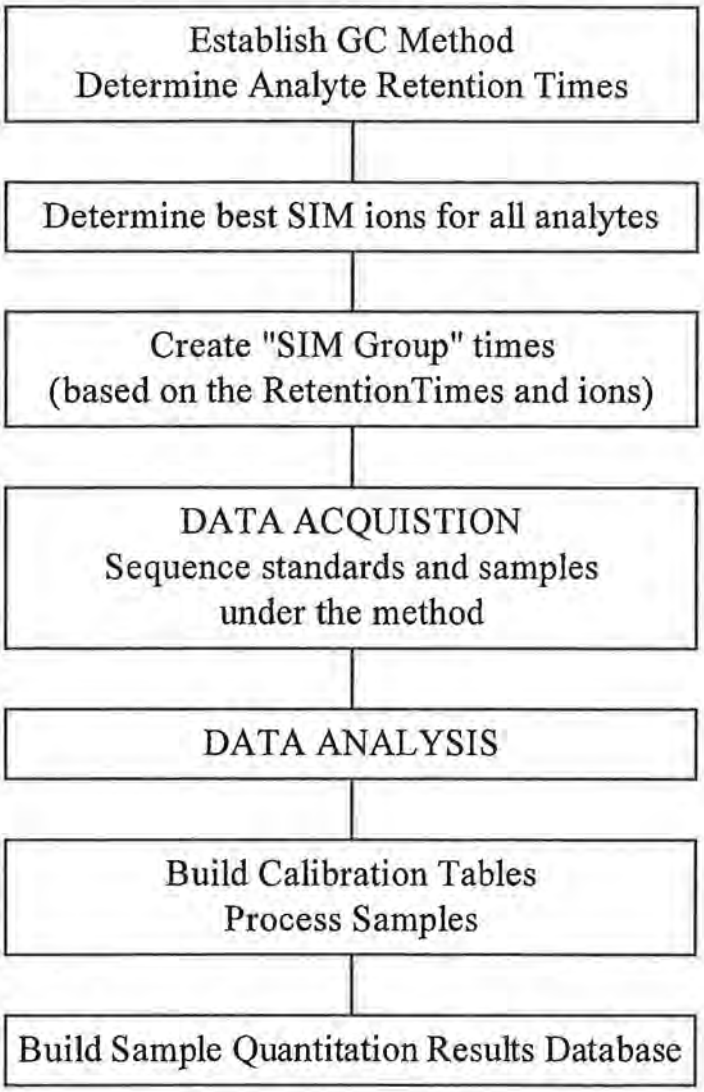

Figure 2-5 Mass Spectrometric Selected-Ion-Monitoring Acquisition and Analysis Overview

GC-MS-SIM Acquisition

Samples were autoinjected by a Hewlett-Packard (Palo Alto, CA) 7973A autosampler into a 60 m DB-5MS column (J&W, Folsom, CA) inside a Hewlett-Packard 5890 series II gas

chromatograph equipped with a Hewlett-Packard 5971A MSD quadrupole mass spectrometer. Two microliters of sample were routinely injected and an electronic pressure programming in either constant flow or with pressure pulse was used during injection as specified by the particular method. Details of the acquisition method such as gas chromatograph oven parameters, SIM groups, ion dwell, etc., can be found in the Analytical Appendix.

Standards: Nominal standard concentrations of 20 pg/μL, 50 pg/μL, 100 pg/μL, 250 pg/μL, 500 pg/μL, 1 ng/μL, 10 ng/μL were injected with all sample sets. Standards were arranged in acquisition sequences to bracket samples.

GC-MS-SIM Data Analysis

Calibration tables were constructed using Hewlett-Packard EnviroQuant software for all analytes based on the relative response of the peak areas to the GC-MS instrumental internal standard. Calibration curves used quadratic, forced origin fitting. This fitting algorithm provided the highest degree of fit and flexibility over the two orders of magnitude represented by the range of standard concentrations. Details of the analysis method such as analyte retention times, examples of analyte response factors, quantitation and confirmation ions, etc., can be found in the Analytical Appendix.

Chapter 3
DATA AND RESULTS

In this chapter experimental results and preliminary interpretations of the data are presented. The objective is to introduce summaries of the data, delineate salient features and trends, and make overall comparisons between SPMDs and oysters in a general and global fashion. More quantitative interpretations will follow in the next chapter.

The following sections provide data on key experimental aspects of each exposure study in tabular form. These are the diluter performance statistics; average oyster wet, dry, and lipid weights; average PAH water sample concentrations taken over the course of the experiment; and the average uncorrected and recovery corrected PAH totals and recovery corrected PAH concentrations in oysters and SPMDs for each day of exposure. The data suggests that the values corrected for surrogate recoveries are more meaningful and unless otherwise indicated, all numbers should be considered recovery corrected[1].

The analytical results for each oyster and SPMD sample, oyster and SPMD control, and SPMD trip blank are compiled in the separate report, Laboratory Comparisons of Oysters and Semipermeable Membrane Devices (SPMDs) - Data Appendix.

Note that 3-methylcholanthrene, an interesting compound in view of its high toxicity, was a troublesome analyte in all respects as it exhibited poor behavior in the diluter, in HRGC-MS-SIM detection and quantitation, etc., making results difficult to interpret.

[1] See the Analytical Appendix for details. Note that the values for PAHs in SPMDs are not corrected for biofouling via the loss of the permeation reference compound, perdeuterated phenanthrene.

10 PPT EXPOSURE

Diluter Performance

Table 3-1 10 ppt Trial Characteristics

Average Dilution Factor	$1.02 \times 10^{-4} \pm 4\%$
Delivered Nominal Concentration	10.2 ppt
Average Temperature	$17.1^{\circ}C \pm 0.8\ ^{\circ}C$

Oyster Characteristics

Table 3-2 10 ppt Study: Oyster tissue characteristics

	Wet weight (g)	% Dry Weight	% Lipid (of wet weight)	% Lipid (of dry weight)
Controls Average	11.13	17.29	1.38	6.88
Controls Standard Deviation	3.83	4.35	0.43	2.35
Exposed Average	7.69	16.13	1.68	8.64
Exposed Standard Deviation	1.68	2.50	0.41	1.14

Water Samples

Table 3-3 10 ppt Trial: Average diluter seawater PAH concentrations (n= 9) as ng/L and percent relative standard deviation.

Compound	Average (ng/L)	% RSD
naphthalene	15.4	7
benzo[b]thiophene	9.5	5
2-methylnaphthalene	11.4	6
1-methylnaphthalene	10.8	2
biphenyl	8.7	2
1-ethylnaphthalene	8.2	5
1,3-dimethylnaphthalene	8.8	6
acenaphthylene	9.1	9
acenaphthene (1,8-hydroacenaphthylene)	8.7	7
4-methylbiphenyl	7.4	18
2,3,5-trimethylnaphthalene	9.5	13
fluorene	10.3	22
1-methylfluorene	9.4	9
dibenzothiophene	8.6	5
phenanthrene	9.2	5
anthracene	6.9	5
2-methylphenanthrene	7.8	6
9-methylanthracene	6.4	9
3,6-dimethylphenanthrene	7.2	8
fluoranthene (1,2-benzacenaphthene)	6.7	6
pyrene	6.6	7
2-methylfluoranthene	6.3	9
benzo[b]naphtho[2,1-d]thiophene	3.4	11
benz[a]anthracene (1,2-benzanthracene)	3.2	9
chrysene	3.8	12
benzo[b]fluoranthene (2,3-benzofluoranthene)	3.1	10
benzo[k]fluoranthene (8,9-benzofluoranthene)	3.0	12
benzo[e]pyrene(4,5-benzopyrene)	3.5	14
benzo[a]pyrene	2.5	17
perylene	2.7	9
3-methylcholanthrene	1.4	29
indeno[1,2,3-c,d]pyrene	2.2	13
dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)	1.8	20
benzo[g,h,i]perylene (1,12-benzoperylene)	2.6	8

Average Total PAH Concentrations in Oysters and SPMDs

Table 3-4 10 ppt Exposure: Oyster average PAH totals

Period	Uncorrected Total (ng per g wet wt.)	% RSD	Corrected Total (ng per g wet wt.)	% RSD
5 Days	315	43	406	56
10 Days	581	10	880	11
15 Days	572	17	821	19
20 Days	1320	22	1490	22
	Uncorrected Total (ng per g dry wt.)	% RSD	Corrected Total (ng per g dry wt.)	% RSD
5 Days	1830	34	2280	38
10 Days	3540	18	5410	25
15 Days	3660	15	5260	18
20 Days	8530	10	9750	20
	Uncorrected Total (ng per g lipid)	% RSD	Corrected Total (ng per g lipid)	% RSD
5 Days	16900	28	21400	36
10 Days	35500	36	55300	45
15 Days	37400	22	54000	28
20 Days	86100	22	99700	34

Table 3-5 10 ppt Exposure: SPMD average PAH totals

Period	Uncorrected Total (ng / SPMD)	% RSD	Corrected Total (ng / SPMD)	RSD
5 Days	1090	16	1520	5
10 Days	2090	3	2940	4
15 Days	3380	6	4200	2
20 Days	3770	13	5000	4

PAH Concentrations in Oysters and SPMDs

Table 3-6 10 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 5 days of exposure.

5 day	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[2]
naphthalene	2.09 (22%)	12.2 (4%)	115 (8%)
benzo[b]thiophene	0 (0%)	0 (0%)	0 (0%)
2-methylnaphthalene	3.21 (25%)	18.8 (10%)	176 (7%)
1-methylnaphthalene	3.96 (23%)	23.2 (12%)	217 (7%)
biphenyl	3.86 (27%)	22.5 (11%)	210 (9%)
1-ethylnaphthalene	5.23 (28%)	30.5 (13%)	285 (10%)
1,3-dimethylnaphthalene	6.07 (30%)	35.2 (13%)	330 (12%)
acenaphthylene	2.27 (29%)	13.2 (12%)	124 (11%)
acenaphthene (1,8-hydroacenaphthylene)	4.93 (37%)	28.4 (20%)	266 (19%)
4-methylbiphenyl	6.27 (59%)	35.2 (41%)	331 (40%)
2,3,5-trimethylnaphthalene	13.3 (29%)	77.7 (20%)	723 (14%)
fluorene (diphenylenemethane)	5.78 (30%)	33.5 (13%)	314 (12%)
1-methylfluorene	10.3 (42%)	58.8 (23%)	552 (24%)
dibenzothiophene	9.42 (33%)	54.3 (16%)	509 (15%)
phenanthrene	11 (31%)	63.5 (14%)	594 (13%)
anthracene	11.3 (40%)	64.9 (22%)	608 (22%)
2-methylphenanthrene	14.6 (46%)	82.5 (27%)	775 (28%)
9-methylanthracene	13.1 (43%)	74.2 (24%)	698 (25%)
3,6-dimethylphenanthrene	19.2 (46%)	108 (28%)	1020 (29%)
fluoranthene (1,2-benzacenaphthene)	23.2 (42%)	132 (24%)	1240 (23%)
pyrene	22.1 (49%)	125 (30%)	1180 (30%)
2-methylfluoranthene	22.8 (57%)	128 (39%)	1200 (38%)
benzo[b]naphtho[2,1-d]thiophene	26.2 (73%)	145 (55%)	1350 (53%)
benz[a]anthracene (1,2-benzanthracene)	22.4 (68%)	124 (50%)	1160 (49%)
chrysene	24.4 (68%)	135 (49%)	1270 (48%)
benzo[b]fluoranthene	20.3 (74%)	112 (56%)	1050 (54%)
benzo[k]fluoranthene	16.7 (78%)	91.7 (60%)	858 (58%)
benzo[e]pyrene (4,5-benzopyrene)	22.5 (66%)	125 (47%)	1170 (46%)
benzo[a]pyrene	11.9 (78%)	65.6 (59%)	612 (58%)
perylene	19.5 (65%)	108 (46%)	1020 (45%)
3-methylcholanthrene	3.91 (155%)	20.6 (141%)	186 (144%)
indeno[1,2,3-c,d]pyrene	7.97 (77%)	43.8 (58%)	408 (57%)
dibenz[a,h]anthracene	3.76 (155%)	19.9 (141%)	179 (144%)
benzo[g,h,i]perylene	12.2 (62%)	67.9 (44%)	636 (43%)

[2] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-7 10 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 10 days of exposure

10 day	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[3]
naphthalene	2.23 (11%)	13.6 (19%)	135 (33%)
benzo[b]thiophene	0 (0%)	0 (0%)	0 (0%)
2-methylnaphthalene	3.51 (33%)	20.6 (21%)	196 (7%)
1-methylnaphthalene	3.96 (28%)	23.5 (19%)	225 (13%)
biphenyl	4.31 (35%)	25.4 (24%)	240 (7%)
1-ethylnaphthalene	6.47 (32%)	38.3 (25%)	364 (13%)
1,3-dimethylnaphthalene	7.74 (30%)	46.0 (24%)	439 (15%)
acenaphthylene	2.03 (80%)	11.3 (76%)	101 (67%)
acenaphthene (1,8-hydroacenaphthylene)	5.47 (40%)	32.1 (32%)	301 (13%)
4-methylbiphenyl	8.83 (25%)	53.1 (23%)	513 (23%)
2,3,5-trimethylnaphthalene	18.7 (22%)	113 (23%)	1100 (27%)
fluorene (diphenylenemethane)	7.26 (31%)	43.0 (22%)	410 (12%)
1-methylfluorene	16.3 (23%)	97.9 (23%)	951 (25%)
dibenzothiophene	13.1 (22%)	78.7 (19%)	764 (22%)
phenanthrene	15.8 (18%)	95.1 (15%)	928 (23%)
anthracene	17.3 (26%)	103 (21%)	992 (19%)
2-methylphenanthrene	26.7 (15%)	162 (19%)	1600 (30%)
9-methylanthracene	23.5 (17%)	142 (14%)	1390 (24%)
3,6-dimethylphenanthrene	42.0 (13%)	260 (29%)	2660 (47%)
fluoranthene (1,2-benzacenaphthene)	41.4 (11%)	253 (22%)	2530 (36%)
pyrene	43.6 (11%)	267 (24%)	2690 (40%)
2-methylfluoranthene	54.1 (15%)	335 (30%)	3460 (50%)
benzo[b]naphtho[2,1-d]thiophene	63.6 (19%)	395 (34%)	4110 (54%)
benz[a]anthracene (1,2-benzanthracene)	55.0 (16%)	340 (29%)	3530 (51%)
chrysene	58.7 (18%)	364 (32%)	3790 (53%)
benzo[b]fluoranthene	55.6 (26%)	347 (39%)	3650 (59%)
benzo[k]fluoranthene	47.1 (28%)	293 (40%)	3090 (59%)
benzo[e]pyrene (4,5-benzopyrene)	58.9 (25%)	366 (38%)	3840 (57%)
benzo[a]pyrene	32.8 (24%)	202 (34%)	2100 (51%)
perylene	48.8 (19%)	302 (32%)	3140 (52%)
3-methylcholanthrene	17.6 (24%)	106 (21%)	1070 (40%)
indeno[1,2,3-c,d]pyrene	24.8 (30%)	154 (41%)	1600 (56%)
dibenz[a,h]anthracene	18.8 (35%)	116 (42%)	1220 (60%)
benzo[g,h,i]perylene	34.2 (29%)	213 (41%)	2210 (57%)

[3] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-8 10 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 15 days of exposure

15 day	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[4]
naphthalene	1.8 (6%)	11.6 (8%)	118 (17%)
benzo[b]thiophene	0 (0%)	0 (0%)	0 (0%)
2-methylnaphthalene	2.94 (16%)	18.9 (18%)	192 (19%)
1-methylnaphthalene	3.33 (11%)	21.4 (10%)	217 (13%)
biphenyl	3.65 (16%)	23.3 (13%)	236 (14%)
1-ethylnaphthalene	5.4 (13%)	34.5 (9%)	350 (10%)
1,3-dimethylnaphthalene	6.61 (13%)	42.2 (8%)	427 (10%)
acenaphthylene	1.82 (20%)	11.6 (19%)	118 (19%)
acenaphthene (1,8-hydroacenaphthylene)	4.41 (17%)	28.2 (15%)	285 (13%)
4-methylbiphenyl	6.97 (12%)	44.5 (7%)	453 (13%)
2,3,5-trimethylnaphthalene	16.8 (7%)	107 (7%)	1090 (13%)
fluorene (diphenylenemethane)	6.12 (15%)	39.2 (11%)	397 (12%)
1-methylfluorene	14.3 (11%)	90.8 (2%)	922 (11%)
dibenzothiophene	11.6 (12%)	73.7 (2%)	747 (9%)
phenanthrene	14 (13%)	89.3 (2%)	907 (10%)
anthracene	14.7 (9%)	95 (11%)	963 (17%)
2-methylphenanthrene	23.9 (18%)	152 (12%)	1550 (18%)
9-methylanthracene	19.7 (11%)	126 (5%)	1280 (14%)
3,6-dimethylphenanthrene	39.5 (22%)	250 (15%)	2550 (19%)
fluoranthene (1,2-benzacenaphthene)	37.1 (14%)	236 (4%)	2400 (12%)
pyrene	39.5 (14%)	251 (6%)	2560 (15%)
2-methylfluoranthene	49.9 (21%)	318 (15%)	3250 (23%)
benzo[b]naphtho[2,1-d]thiophene	58.2 (28%)	371 (26%)	3830 (36%)
benz[a]anthracene (1,2-benzanthracene)	50.3 (26%)	320 (24%)	3300 (33%)
chrysene	53.8 (27%)	343 (25%)	3540 (34%)
benzo[b]fluoranthene	51.7 (26%)	331 (24%)	3420 (35%)
benzo[k]fluoranthene	45.7 (27%)	292 (26%)	3020 (36%)
benzo[e]pyrene (4,5-benzopyrene)	55.2 (23%)	354 (23%)	3650 (33%)
benzo[a]pyrene	32.8 (31%)	211 (31%)	2190 (42%)
perylene	46.3 (29%)	296 (27%)	3060 (38%)
3-methylcholanthrene	17.8 (37%)	114 (37%)	1190 (48%)
indeno[1,2,3-c,d]pyrene	28.7 (34%)	184 (33%)	1910 (44%)
dibenz[a,h]anthracene	22.1 (31%)	142 (32%)	1480 (43%)
benzo[g,h,i]perylene	36 (27%)	232 (28%)	2400 (39%)

[4] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-9 10 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 20 days of exposure

20 day	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[5]
naphthalene	0.915 (110%)	5.81 (115%)	53.7 (115%)
benzo[b]thiophene	0 (0%)	0 (0%)	0 (0%)
2-methylnaphthalene	3.26 (28%)	20.9 (14%)	207 (7%)
1-methylnaphthalene	3.56 (21%)	23.1 (10%)	229 (8%)
biphenyl	3.77 (29%)	24.1 (14%)	240 (13%)
1-ethylnaphthalene	6.19 (29%)	39.6 (13%)	394 (13%)
1,3-dimethylnaphthalene	7.52 (29%)	48.0 (14%)	478 (14%)
acenaphthylene	1.04 (114%)	6.15 (110%)	62.5 (111%)
acenaphthene (1,8-hydroacenaphthylene)	4.46 (30%)	28.5 (14%)	283 (14%)
4-methylbiphenyl	8.54 (32%)	54.4 (17%)	542 (17%)
2,3,5-trimethylnaphthalene	25.2 (30%)	161 (16%)	1600 (13%)
fluorene (diphenylenemethane)	6.74 (30%)	43.0 (15%)	428 (14%)
1-methylfluorene	18.0 (25%)	116 (11%)	1150 (15%)
dibenzothiophene	14.5 (27%)	93.1 (12%)	930 (13%)
phenanthrene	18 (25%)	116 (11%)	1150 (11%)
anthracene	17.7 (27%)	113 (16%)	1130 (18%)
2-methylphenanthrene	36.7 (20%)	238 (12%)	2390 (19%)
9-methylanthracene	29.2 (29%)	187 (20%)	1880 (26%)
3,6-dimethylphenanthrene	71.3 (12%)	467 (10%)	4710 (22%)
fluoranthene (1,2-benzacenaphthene)	56.9 (14%)	371 (6%)	3720 (15%)
pyrene	63.6 (14%)	415 (7%)	4170 (17%)
2-methylfluoranthene	95.3 (14%)	625 (16%)	6350 (28%)
benzo[b]naphtho[2,1-d]thiophene	114 (25%)	752 (30%)	7720 (41%)
benz[a]anthracene (1,2-benzanthracene)	101 (23%)	663 (27%)	6790 (38%)
chrysene	102 (23%)	672 (27%)	6880 (38%)
benzo[b]fluoranthene	112 (29%)	734 (30%)	7570 (43%)
benzo[k]fluoranthene	97.7 (31%)	642 (32%)	6650 (46%)
benzo[e]pyrene (4,5-benzopyrene)	121 (29%)	799 (32%)	8260 (44%)
benzo[a]pyrene	63.6 (33%)	410 (26%)	4220 (39%)
perylene	89.3 (31%)	584 (31%)	6040 (45%)
3-methylcholanthrene	28.5 (51%)	181 (43%)	1880 (56%)
indeno[1,2,3-c,d]pyrene	52.7 (39%)	341 (35%)	3540 (48%)
dibenz[a,h]anthracene	45.7 (42%)	295 (38%)	3080 (54%)
benzo[g,h,i]perylene	74.7 (33%)	487 (31%)	5050 (46%)

[5] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-10 10 ppt Study: Average PAH concentrations accumulated in SPMDs as ng/SPMD and relative standard deviation (RSD) as percent

Compound	5 day	10 day	15 day	20 day
naphthalene	24.2 (67%)*	42 (2%)	41.4 (1%)	35.2 (1%)
benzo[b]thiophene	nd	nd	nd	nd
2-methylnaphthalene	49.8 (2%)	78.6 (4%)	88.7 (4%)	98.8 (1%)
1-methylnaphthalene	54.5 (2%)	76.9 (3%)	90.4 (4%)	99.4 (1%)
biphenyl	47.8 (2%)	76.6 (3%)	95.7 (3%)	106 (1%)
1-ethylnaphthalene	56.7 (4%)	97.1 (1%)	132 (5%)	153 (2%)
1,3-dimethylnaphthalene	64.9 (12%)	110 (1%)	152 (5%)	182 (2%)
acenaphthylene	39.4 (16%)	54.6 (4%)	68.5 (4%)	79.9 (2%)
acenaphthene (1,8-hydroacenaphthylene)	52.1 (2%)	85.3 (5%)	111 (6%)	126 (3%)
4-methylbiphenyl	62.2 (4%)	105 (6%)	141 (6%)	171 (5%)
2,3,5-trimethylnaphthalene	65.6 (10%)	124 (2%)	185 (2%)	222 (2%)
fluorene (diphenylenemethane)	55.4 (2%)	98 (2%)	134 (2%)	152 (2%)
1-methylfluorene	61 (3%)	116 (3%)	177 (1%)	202 (4%)
dibenzothiophene	59.3 (3%)	115 (2%)	161 (2%)	185 (3%)
phenanthrene	72.4 (4%)	130 (2%)	183 (1%)	205 (3%)
anthracene	59.2 (3%)	111 (3%)	167 (0%)	190 (3%)
2-methylphenanthrene	67.3 (3%)	131 (4%)	196 (2%)	228 (4%)
9-methylanthracene	66.7 (3%)	137 (8%)	205 (2%)	241 (3%)
3,6-dimethylphenanthrene	63.3 (2%)	130 (5%)	193 (3%)	226 (5%)
fluoranthene (1,2-benzacenaphthene)	60.9 (3%)	122 (4%)	194 (2%)	224 (5%)
pyrene	63 (3%)	126 (6%)	192 (3%)	221 (6%)
2-methylfluoranthene	56.3 (3%)	123 (6%)	194 (3%)	227 (7%)
benzo[b]naphtho[2,1-d]thiophene	53 (6%)	107 (7%)	161 (3%)	192 (7%)
benz[a]anthracene (1,2-benzanthracene)	52.9 (3%)	101 (4%)	156 (3%)	185 (7%)
chrysene	53.9 (3%)	93.6 (16%)	120 (7%)	170 (9%)
benzo[b]fluoranthene (2,3-benzofluoranthene)	39.7 (3%)	73 (15%)	94.5 (7%)	134 (11%)
benzo[k]fluoranthene (8,9-benzofluoranthene)	35.3 (4%)	53.7 (12%)	67.8 (9%)	99.1 (12%)
benzo[e]pyrene (4,5-benzopyrene)	42.5 (3%)	75.5 (16%)	86.6 (15%)	128 (12%)
benzo[a]pyrene	32.2 (4%)	63.4 (9%)	84.3 (6%)	119 (11%)
perylene	35.8 (4%)	70 (4%)	109 (4%)	130 (11%)
3-methylcholanthrene	nd	41.7 (9%)	78.8 (5%)	95.2 (11%)
indeno[1,2,3-c,d]pyrene	nd	33.9 (4%)	55 (5%)	67.2 (14%)
dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)	nd	nd	39.5 (8%)	47 (14%)
benzo[g,h,i]perylene (1,12-benzoperylene)	nd	35.8 (4%)	54.5 (6%)	65 (12%)

* includes NDs

100 PPT EXPOSURE

Diluter Performance

Table 3-11 100 ppt Study Characteristics

Average Dilution Factor	$1.01 \times 10^{-4} \pm 4\%$
Delivered Nominal Concentration	101 ppt
Average Temperature	$16.4^{\circ}C \pm 1.2\ ^{\circ}C$

Oyster Characteristics

Table 3-12 100 ppt Study: Oyster tissue characteristics

	Wet weight (g)	% Dry Weight	% Lipid (of wet weight)	% Lipid (of dry weight)
Controls Average	16.29	13.88	1.13	6.99
Controls Standard Deviation	2.83	1.46	0.29	1.73
Exposed Averages	8.81	14.22	1.64	9.82
Exposed Standard Deviation	1.38	2.02	0.46	1.98

Water Samples

Table 3-13 100 ppt Study: Average diluter seawater PAH concentrations (n = 9) as ng/L and percent relative standard deviation (%RSD).

Compound	Average	% RSD
naphthalene	100	1
benzo[b]thiophene	95	2
2-methylnaphthalene	95	2
1-methylnaphthalene	94	2
biphenyl	89	2
1-ethylnaphthalene	87	3
1,3-dimethylnaphthalene	80	5
acenaphthylene	85	5
acenaphthene (1,8-hydroacenaphthylene)	82	1
4-methylbiphenyl	76	5
2,3,5-trimethylnaphthalene	85	7
fluorene (diphenylenemethane)	92	2
1-methylfluorene	91	6
dibenzothiophene	88	3
phenanthrene	87	4
anthracene	76	7
2-methylphenanthrene	80	10
9-methylanthracene	67	12
3,6-dimethylphenanthrene	77	15
fluoranthene (1,2-benzacenaphthene)	75	8
pyrene	74	9
2-methylfluoranthene	71	13
benzo[b]naphtho[2,1-d]thiophene	52	23
benz[a]anthracene (1,2-benzanthracene)	48	22
chrysene	57	25
benzo[b]fluoranthene (2,3-benzofluoranthene)	44	28
benzo[k]fluoranthene (8,9-benzofluoranthene)	43	29
benzo[e]pyrene	45	30
benzo[a]pyrene	38	32
perylene	42	28
3-methylcholanthrene	20	41
indeno[1,2,3-c,d]pyrene	31	34
dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)	28	34
benzo[g,h,i]perylene (1,12-benzoperylene)	35	33

Average Total PAH Concentrations in Oysters and SPMDs

Table 3-14 100 ppt Exposure: Oyster average PAH totals

Period	Uncorrected Amount (ng per g wet wt)	% RSD	Corrected Amount (ng per g wet wt)	% RSD
5 Days	2470	22	3120	23
10 Days	2550	43	3200	41
15 Days	4630	20	6210	18
20 Days	6360	36	8100	33
	Uncorrected Amount (ng per g dry wt.)	% RSD	Corrected Amount (ng per g dry wt)	% RSD
5 Days	16400	28	20700	28
10 Days	18300	42	22900	39
15 Days	35500	20	47500	19
20 Days	43900	26	56100	23
	Uncorrected Amount (ng per g lipid)	% RSD	Corrected Amount (ng per g lipid)	% RSD
5 Days	177000	36	222000	36
10 Days	169000	44	212000	41
15 Days	367000	24	492000	24
20 Days	310000	26	397000	24

Table 3-15 100 ppt Exposure: SPMD average PAH totals

Period	Uncorrected Amount (ng / SPMD)	% RSD	Corrected Amount (ng / SPMD)	% RSD
5 Days	9190	12	11500	4
10 Days	19800	19	27800	9
15 Days	15400	8	38700	5
20 Days	20000	4	50300	4

Table 3-16 100 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 5 days of exposure

5 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[6]
naphthalene	8.68 (44%)	52.6 (33%)	522 (18%)
benzo[b]thiophene	5.87 (25%)	33.9 (18%)	315 (4%)
2-methylnaphthalene	20.6 (39%)	132 (25%)	1370 (15%)
1-methylnaphthalene	20.9 (37%)	134 (23%)	1390 (14%)
biphenyl	24.4 (34%)	156 (21%)	1630 (17%)
1-ethylnaphthalene	35.6 (29%)	230 (19%)	2440 (21%)
1,3-dimethylnaphthalene	44.6 (26%)	290 (18%)	3080 (23%)
acenaphthylene	15.4 (43%)	97.3 (29%)	1010 (18%)
acenaphthene (1,8-hydroacenaphthylene)	26.3 (33%)	169 (21%)	1770 (18%)
4-methylbiphenyl	43.3 (22%)	281 (11%)	2990 (17%)
2,3,5-trimethylnaphthalene	74.1 (20%)	488 (20%)	5260 (29%)
fluorene (diphenylenemethane)	30.9 (27%)	200 (17%)	2120 (19%)
1-methylfluorene	91.3 (20%)	600 (20%)	6440 (28%)
dibenzothiophene	73.1 (21%)	479 (18%)	5140 (26%)
phenanthrene	81.6 (20%)	536 (20%)	5740 (28%)
anthracene	82.5 (21%)	541 (19%)	5770 (26%)
2-methylphenanthrene	149 (18%)	992 (25%)	10700 (35%)
9-methylanthracene	108 (22%)	716 (24%)	7680 (32%)
3,6-dimethylphenanthrene	222 (20%)	1480 (29%)	16100 (38%)
fluoranthene (1,2-benzacenaphthene)	171 (17%)	1130 (25%)	12300 (35%)
pyrene	184 (18%)	1230 (26%)	13300 (36%)
2-methylfluoranthene	257 (20%)	1710 (29%)	18500 (39%)
benzo[b]naphtho[2,1-d]thiophene	177 (33%)	1180 (41%)	12700 (49%)
benz[a]anthracene (1,2-benzanthracene)	159 (35%)	1060 (43%)	11400 (50%)
chrysene	168 (32%)	1120 (39%)	12100 (47%)
benzo[b]fluoranthene	168 (31%)	1120 (38%)	12000 (46%)
benzo[k]fluoranthene	138 (34%)	918 (40%)	9840 (46%)
benzo[e]pyrene (4,5-benzopyrene)	134 (29%)	891 (36%)	9600 (44%)
benzo[a]pyrene	102 (39%)	675 (43%)	7190 (48%)
perylene	81.3 (35%)	540 (39%)	5770 (46%)
3-methylcholanthrene	50.1 (53%)	327 (51%)	3390 (51%)
indeno[1,2,3-c,d]pyrene	55.6 (34%)	367 (38%)	3910 (42%)
dibenz(a,h)anthracene	60.5 (40%)	405 (47%)	4340 (54%)
benzo[g,h,i]perylene	59.8 (34%)	399 (40%)	4280 (47%)

[6] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-17 100 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 10 days of exposure

10 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[7]
naphthalene	9 (28%)	62.9 (12%)	578 (10%)
benzo[b]thiophene	5 (28%)	34.9 (12%)	321 (11%)
2-methylnaphthalene	26.4 (29%)	184 (14%)	1690 (12%)
1-methylnaphthalene	25.8 (27%)	180 (11%)	1650 (9%)
biphenyl	31.7 (26%)	221 (11%)	2030 (9%)
1-ethylnaphthalene	48 (25%)	336 (11%)	3090 (10%)
1,3-dimethylnaphthalene	61 (24%)	428 (11%)	3930 (10%)
acenaphthylene	20.7 (29%)	144 (14%)	1330 (12%)
acenaphthene (1,8-hydroacenaphthylene)	35 (25%)	246 (9%)	2260 (7%)
4-methylbiphenyl	60 (22%)	427 (18%)	3940 (20%)
2,3,5-trimethylnaphthalene	101 (23%)	717 (17%)	6600 (18%)
fluorene (diphenylenemethane)	41.2 (24%)	289 (10%)	2660 (8%)
1-methylfluorene	121 (23%)	859 (17%)	7910 (19%)
dibenzothiophene	94.7 (22%)	669 (12%)	6150 (13%)
phenanthrene	102 (23%)	724 (14%)	6660 (15%)
anthracene	104 (20%)	741 (15%)	6830 (16%)
2-methylphenanthrene	177 (36%)	1270 (35%)	11700 (36%)
9-methylanthracene	143 (24%)	1020 (22%)	9390 (24%)
3,6-dimethylphenanthrene	251 (43%)	1810 (42%)	16700 (43%)
fluoranthene (1,2-benzacenaphthene)	197 (30%)	1410 (30%)	13000 (32%)
pyrene	208 (33%)	1500 (32%)	13800 (34%)
2-methylfluoranthene	266 (48%)	1920 (47%)	17800 (49%)
benzo[b]naphtho[2,1-d]thiophene	143 (66%)	1030 (65%)	9560 (67%)
benz[a]anthracene (1,2-benzanthracene)	135 (64%)	972 (62%)	9030 (64%)
chrysene	139 (66%)	1000 (65%)	9280 (67%)
benzo[b]fluoranthene	136 (74%)	979 (72%)	9090 (74%)
benzo[k]fluoranthene	103 (74%)	744 (72%)	6880 (74%)
benzo[e]pyrene (4,5-benzopyrene)	108 (71%)	780 (69%)	7230 (71%)
benzo[a]pyrene	82.1 (70%)	590 (68%)	5450 (70%)
perylene	63.8 (63%)	458 (62%)	4240 (63%)
3-methylcholanthrene	43.1 (69%)	309 (67%)	2850 (69%)
indeno[1,2,3-c,d]pyrene	38.6 (72%)	278 (70%)	2570 (72%)
dibenz(a,h)anthracene	38 (81%)	273 (79%)	2530 (81%)
benzo[g,h,i]perylene	44.4 (68%)	319 (67%)	2950 (69%)

[7] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-18 100 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 15 days of exposure.

15 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[8]
naphthalene	7.18 (15%)	54.5 (9%)	558 (5%)
benzo[b]thiophene	3.8 (18%)	29 (10%)	304 (6%)
2-methylnaphthalene	21.7 (15%)	165 (9%)	1690 (5%)
1-methylnaphthalene	21 (16%)	159 (10%)	1630 (5%)
biphenyl	26.8 (14%)	203 (8%)	2080 (5%)
1-ethylnaphthalene	47.3 (17%)	359 (11%)	3670 (6%)
1,3-dimethylnaphthalene	62 (16%)	471 (10%)	4820 (6%)
acenaphthylene	15.8 (16%)	120 (10%)	1230 (7%)
acenaphthene (1,8-hydroacenaphthylene)	29.8 (15%)	226 (9%)	2320 (6%)
4-methylbiphenyl	65.4 (15%)	497 (9%)	5090 (6%)
2,3,5-trimethylnaphthalene	133 (14%)	1000 (8%)	10300 (7%)
fluorene (diphenylenemethane)	39.3 (15%)	299 (8%)	3060 (6%)
1-methylfluorene	158 (14%)	1200 (8%)	12300 (8%)
dibenzothiophene	111 (14%)	847 (7%)	8680 (7%)
phenanthrene	128 (14%)	974 (8%)	10000 (8%)
anthracene	135 (13%)	1030 (7%)	10500 (9%)
2-methylphenanthrene	304 (15%)	2310 (10%)	23700 (11%)
9-methylanthracene	240 (15%)	1830 (12%)	18900 (16%)
3,6-dimethylphenanthrene	510 (19%)	3890 (19%)	40200 (22%)
fluoranthene (1,2-benzacenaphthene)	343 (15%)	2610 (13%)	26900 (16%)
pyrene	384 (15%)	2930 (14%)	30200 (18%)
2-methylfluoranthene	585 (22%)	4480 (23%)	46400 (27%)
benzo[b]naphtho[2,1-d]thiophene	365 (25%)	2800 (27%)	29100 (32%)
benz[a]anthracene (1,2-benzanthracene)	341 (26%)	2610 (27%)	27000 (30%)
chrysene	340 (25%)	2610 (27%)	27100 (31%)
benzo[b]fluoranthene	357 (33%)	2750 (36%)	28700 (41%)
benzo[k]fluoranthene	281 (30%)	2160 (32%)	22600 (37%)
benzo[e]pyrene (4,5-benzopyrene)	278 (29%)	2140 (32%)	22300 (36%)
benzo[a]pyrene	219 (29%)	1680 (32%)	17600 (38%)
perylene	180 (29%)	1380 (32%)	14400 (37%)
3-methylcholanthrene	116 (24%)	896 (29%)	9390 (39%)
indeno[1,2,3-c,d]pyrene	119 (31%)	915 (34%)	9570 (39%)
dibenz(a,h)anthracene	120 (32%)	920 (35%)	9590 (39%)
benzo[g,h,i]perylene	125 (28%)	964 (31%)	10000 (37%)

[8] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-19 100 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 20 days of exposure.

20 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[9]
naphthalene	9.34 (17%)	67.1 (9%)	478 (17%)
benzo[b]thiophene	5.41 (15%)	37.4 (9%)	263 (18%)
2-methylnaphthalene	29.1 (18%)	203 (9%)	1450 (16%)
1-methylnaphthalene	29 (18%)	202 (9%)	1440 (15%)
biphenyl	36.8 (18%)	257 (9%)	1830 (15%)
1-ethylnaphthalene	62.5 (20%)	434 (8%)	3080 (13%)
1,3-dimethylnaphthalene	83 (23%)	576 (12%)	4090 (15%)
acenaphthylene	22.2 (22%)	155 (12%)	1100 (18%)
acenaphthene (1,8-hydroacenaphthylene)	41.1 (18%)	287 (9%)	2050 (16%)
4-methylbiphenyl	94.1 (21%)	656 (11%)	4650 (14%)
2,3,5-trimethylnaphthalene	169 (28%)	1170 (17%)	8280 (18%)
fluorene (diphenylenemethane)	53 (19%)	369 (9%)	2630 (15%)
1-methylfluorene	204 (24%)	1420 (13%)	10000 (15%)
dibenzothiophene	142 (20%)	990 (9%)	7020 (12%)
phenanthrene	160 (20%)	1110 (9%)	7860 (11%)
anthracene	179 (24%)	1250 (15%)	8860 (18%)
2-methylphenanthrene	377 (28%)	2610 (17%)	18500 (17%)
9-methylanthracene	294 (26%)	2040 (16%)	14400 (17%)
3,6-dimethylphenanthrene	610 (30%)	4210 (20%)	29700 (19%)
fluoranthene (1,2-benzacenaphthene)	435 (28%)	3010 (18%)	21300 (18%)
pyrene	490 (29%)	3400 (19%)	24000 (19%)
2-methylfluoranthene	729 (31%)	5040 (22%)	35600 (21%)
benzo[b]naphtho[2,1-d]thiophene	493 (34%)	3410 (26%)	24200 (26%)
benz[a]anthracene (1,2-benzanthracene)	460 (35%)	3180 (26%)	22500 (26%)
chrysene	447 (38%)	3090 (28%)	21900 (28%)
benzo[b]fluoranthene	474 (41%)	3270 (31%)	23200 (31%)
benzo[k]fluoranthene	369 (46%)	2540 (36%)	18000 (36%)
benzo[e]pyrene (4,5-benzopyrene)	374 (44%)	2580 (34%)	18200 (35%)
benzo[a]pyrene	300 (45%)	2070 (35%)	14700 (36%)
perylene	243 (40%)	1690 (31%)	11900 (31%)
3-methylcholanthrene	165 (54%)	1130 (44%)	8070 (45%)
indeno[1,2,3-c,d]pyrene	169 (45%)	1170 (36%)	8320 (37%)
dibenz(a,h)anthracene	176 (48%)	1210 (38%)	8630 (39%)
benzo[g,h,i]perylene	178 (43%)	1230 (35%)	8740 (35%)

[9] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-20 100 ppt Study: Average PAH concentrations accumulated in SPMDs as ng/SPMD and relative standard deviation (RSD) as percent

Compounds	5 day	10 day	15 day	20 day
naphthalene	195 (8%)	304 (5%)	325 (3%)	333 (2%)
benzo[b]thiophene	97.3 (1%)	162 (2%)	168 (4%)	166 (1%)
2-methylnaphthalene	355 (2%)	725 (3%)	893 (2%)	1010 (1%)
1-methylnaphthalene	342 (2%)	687 (3%)	848 (2%)	961 (1%)
biphenyl	349 (2%)	743 (3%)	944 (2%)	1100 (1%)
1-ethylnaphthalene	453 (2%)	996 (3%)	1300 (2%)	1620 (2%)
1,3-dimethylnaphthalene	511 (2%)	1140 (4%)	1480 (3%)	1890 (3%)
acenaphthylene	300 (4%)	698 (4%)	833 (2%)	952 (1%)
acenaphthene (1,8-hydroacenaphthylene)	413 (2%)	879 (4%)	1150 (2%)	1360 (1%)
4-methylbiphenyl	475 (2%)	1100 (4%)	1470 (3%)	1880 (3%)
2,3,5-trimethylnaphthalene	567 (4%)	1310 (5%)	1720 (5%)	2310 (5%)
fluorene (diphenylenemethane)	427 (3%)	981 (5%)	1330 (2%)	1620 (1%)
1-methylfluorene	506 (5%)	1200 (5%)	1640 (6%)	2190 (4%)
dibenzothiophene	424 (3%)	1070 (6%)	1510 (4%)	1900 (4%)
phenanthrene	431 (3%)	1100 (8%)	1610 (4%)	2000 (4%)
anthracene	413 (4%)	1070 (8%)	1530 (5%)	1980 (5%)
2-methylphenanthrene	485 (5%)	1240 (8%)	1770 (6%)	2370 (6%)
9-methylanthracene	523 (5%)	1280 (7%)	1850 (6%)	2480 (6%)
3,6-dimethylphenanthrene	517 (6%)	1280 (8%)	1840 (7%)	2470 (7%)
fluoranthene (1,2-benzacenaphthene)	433 (7%)	1160 (8%)	1660 (6%)	2210 (5%)
pyrene	445 (6%)	1170 (9%)	1690 (7%)	2210 (6%)
2-methylfluoranthene	484 (7%)	1240 (10%)	1750 (7%)	2330 (6%)
benzo[b]naphtho[2,1-d]thiophene	292 (6%)	793 (18%)	1210 (7%)	1670 (6%)
1,2-benzanthracene	286 (7%)	784 (17%)	1180 (7%)	1640 (6%)
chrysene	184 (15%)	634 (37%)	1030 (6%)	1240 (7%)
benzo[b]fluoranthene (2,3-benzofluoranthene)	194 (14%)	606 (35%)	977 (8%)	1200 (7%)
benzo[k]fluoranthene (8,9-benzofluoranthene)	129 (13%)	429 (41%)	684 (8%)	855 (7%)
benzo[e]pyrene	318 (23%)	581 (24%)	853 (8%)	1230 (8%)
benzo[a]pyrene	150 (14%)	462 (38%)	754 (9%)	946 (7%)
perylene	196 (8%)	486 (19%)	700 (6%)	1020 (5%)
3-methylcholanthrene	193 (11%)	454 (22%)	624 (7%)	995 (7%)
indeno[1,2,3-c,d]pyrene	149 (11%)	373 (28%)	525 (8%)	804 (9%)
dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)	107 (16%)	283 (22%)	371 (6%)	608 (11%)
benzo[g,h,i]perylene (1,12-benzoperylene)	135 (9%)	336 (24%)	472 (7%)	720 (8%)

250 PPT EXPOSURE

Diluter Performance

Table 3-21 250 ppt Study Characteristics

Average Dilution Factor	$1.01 \times 10^{-4} \pm 4\%$
Delivered Nominal Concentration	252 ppt
Average Temperature	15.8°C ± 1 °C

Oyster Characteristics

Table 3-22 250 ppt Study: Oyster tissue characteristics

	Wet weight (g)	% Dry Weight	% Lipid (of wet weight)	% Lipid (of dry weight)
Controls Average	15.46	13.77	1.21	7.59
Controls Standard Deviation	2.95	2.03	0.28	1.85
Exposed Averages	7.26	14.40	1.55	9.31
Exposed Standard Deviation	1.77	2.54	0.43	2.82

Water Samples

Table 3-23 250 ppt Study: Average diluter seawater PAH concentrations (n=13) as ng/L and percent relative standard deviation (%RSD)

Compound	Average	% RSD
naphthalene	230	5
benzo[b]thiophene	229	5
2-methylnaphthalene	218	6
1-methylnaphthalene	222	5
biphenyl	216	5
1-ethylnaphthalene	210	5
1,3-dimethylnaphthalene	214	6
acenaphthylene	231	7
acenaphthene (1,8-hydroacenaphthylene)	220	5
4-methylbiphenyl	215	4
2,3,5-trimethylnaphthalene	219	5
fluorene (diphenylenemethane)	212	6
1-methylfluorene	212	8
dibenzothiophene	202	6
phenanthrene	200	7
anthracene	184	12
2-methylphenanthrene	196	11
9-methylanthracene	153	9
3,6-dimethylphenanthrene	190	11
fluoranthene (1,2-benzacenaphthene)	181	11
pyrene	180	13
2-methylfluoranthene	179	17
benzo[b]naphtho[2,1-d]thiophene	134	32
benz[a]anthracene (1,2-benzanthracene)	133	32
chrysene	148	35
benzo[b]fluoranthene (2,3-benzofluoranthene)	131	36
benzo[k]fluoranthene (8,9-benzofluoranthene)	132	39
benzo[e]pyrene	133	37
benzo[a]pyrene	117	41
perylene	116	39
3-methylcholanthrene	33	63
indeno[1,2,3-c,d]pyrene	109	43
dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)	102	46
benzo[g,h,i]perylene (1,12-benzoperylene)	110	41

Average Total PAH concentrations in Oysters and SPMDs

Table 3-24 250 ppt Exposure: Oyster average PAH totals

Period	Uncorrected Amount (ng per g wet wt.)	% RSD	Corrected Amount (ng per g wet wt.)	% RSD
5 Days	5430	36	7620	29
10 Days	8550	67	12300	52
15 Days	12200	33	14900	30
20 Days	14300	30	18000	27
	Uncorrected Amount (ng per g dry wt.)	% RSD	Corrected Amount (ng per g dry wt.)	% RSD
5 Days	36800	19	52100	12
10 Days	60900	74	84100	52
15 Days	83400	46	101000	43
20 Days	107000	32	134000	29
	Uncorrected Amount (ng per g lipid)	% RSD	Corrected Amount (ng per g lipid)	% RSD
5 Days	320000	38	445000	32
10 Days	628000	83	831000	61
15 Days	801000	53	967000	48
20 Days	1090000	26	1370000	23

Table 3-25 250 ppt Exposure: SPMD average PAH totals

Period	Uncorrected Totals (ng/SPMD)	% RSD	Corrected Totals (ng/SPMD)	% RSD
5 Days	21600	10	32100	7
10 Days	42000	22	59100	11
15 Days	72400	13	87700	9
20 Days	104000	10	123000	6

PAH concentrations in Oysters and SPMDs

Table 3-26 250 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 5 days of exposure.

5 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[10]
naphthalene	23.6 (37%)	158 (18%)	1400 (41%)
benzo[b]thiophene	16.9 (14%)	98.3 (11%)	1000 (7%)
2-methylnaphthalene	71.1 (39%)	475 (21%)	4200 (41%)
1-methylnaphthalene	69.2 (36%)	467 (18%)	4100 (39%)
biphenyl	94.2 (35%)	636 (18%)	5560 (39%)
1-ethylnaphthalene	148 (36%)	1000 (20%)	8730 (39%)
1,3-dimethylnaphthalene	180 (35%)	1220 (20%)	10600 (38%)
acenaphthylene	63.5 (38%)	426 (20%)	3760 (41%)
acenaphthene (1,8-hydroacenaphthylene)	114 (36%)	769 (19%)	6720 (39%)
4-methylbiphenyl	195 (34%)	1310 (18%)	11500 (39%)
2,3,5-trimethylnaphthalene	313 (33%)	2140 (19%)	18400 (36%)
fluorene (diphenylenemethane)	149 (35%)	1010 (18%)	8780 (38%)
1-methylfluorene	311 (32%)	2120 (17%)	18300 (35%)
dibenzothiophene	261 (33%)	1770 (18%)	15300 (37%)
phenanthrene	279 (33%)	1900 (18%)	16400 (37%)
anthracene	274 (32%)	1860 (16%)	16100 (36%)
2-methylphenanthrene	458 (30%)	3140 (16%)	26900 (35%)
9-methylanthracene	333 (54%)	2150 (46%)	20200 (55%)
3,6-dimethylphenanthrene	681 (27%)	4690 (14%)	39900 (32%)
fluoranthene (1,2-benzacenaphthene)	486 (28%)	3340 (14%)	28500 (33%)
pyrene	509 (28%)	3490 (13%)	29800 (33%)
2-methylfluoranthene	680 (24%)	4700 (12%)	39600 (29%)
benzo[b]naphtho[2,1-d]thiophene	268 (20%)	1870 (15%)	15500 (25%)
benz[a]anthracene (1,2-benzanthracene)	270 (23%)	1880 (15%)	15600 (26%)
chrysene	199 (24%)	1390 (17%)	11500 (27%)
benzo[b]fluoranthene	277 (24%)	1930 (19%)	16000 (26%)
benzo[k]fluoranthene	160 (27%)	1120 (24%)	9190 (28%)
benzo[e]pyrene (4,5-benzopyrene)	201 (26%)	1400 (16%)	11600 (28%)
benzo[a]pyrene	132 (35%)	905 (21%)	7640 (37%)
perylene	103 (34%)	706 (18%)	6000 (36%)
3-methylcholanthrene	18.4 (17%)	108 (20%)	1130 (37%)
indeno[1,2,3-c,d]pyrene	110 (34%)	787 (41%)	6210 (28%)
dibenz(a,h)anthracene	76.9 (42%)	542 (44%)	4300 (37%)
benzo[g,h,i]perylene	104 (29%)	738 (35%)	5860 (23%)

[10] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-27 250 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 10 days of exposure.

10 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[11]
naphthalene	22.4 (54%)	149 (35%)	1410 (15%)
benzo[b]thiophene	14.8 (53%)	95 (33%)	866 (11%)
2-methylnaphthalene	73.5 (50%)	489 (31%)	4640 (10%)
1-methylnaphthalene	68.4 (51%)	454 (32%)	4310 (11%)
biphenyl	98.9 (50%)	658 (31%)	6240 (9%)
1-ethylnaphthalene	164 (47%)	1090 (28%)	10400 (6%)
1,3-dimethylnaphthalene	206 (47%)	1380 (27%)	13100 (5%)
acenaphthylene	65 (56%)	429 (37%)	4040 (16%)
acenaphthene (1,8-hydroacenaphthylene)	119 (50%)	794 (31%)	7530 (9%)
4-methylbiphenyl	230 (41%)	1560 (24%)	14900 (11%)
2,3,5-trimethylnaphthalene	397 (36%)	2690 (20%)	26100 (13%)
fluorene (diphenylenemethane)	166 (44%)	1110 (25%)	10600 (4%)
1-methylfluorene	394 (35%)	2680 (20%)	26000 (17%)
dibenzothiophene	312 (40%)	2100 (22%)	20200 (8%)
phenanthrene	346 (38%)	2340 (21%)	22600 (13%)
anthracene	359 (38%)	2450 (26%)	23800 (26%)
2-methylphenanthrene	671 (41%)	4570 (33%)	44600 (36%)
9-methylanthracene	600 (43%)	4040 (29%)	39000 (24%)
3,6-dimethylphenanthrene	1120 (47%)	7670 (45%)	75700 (52%)
fluoranthene (1,2-benzacenaphthene)	729 (40%)	4980 (34%)	48700 (38%)
pyrene	804 (44%)	5480 (38%)	53600 (43%)
2-methylfluoranthene	1170 (54%)	8060 (55%)	80000 (64%)
benzo[b]naphtho[2,1-d]thiophene	541 (81%)	3770 (88%)	38100 (99%)
benz[a]anthracene (1,2-benzanthracene)	553 (79%)	3850 (87%)	38900 (97%)
chrysene	408 (85%)	2840 (92%)	28800 (103%)
benzo[b]fluoranthene	628 (94%)	4400 (102%)	44600 (113%)
benzo[k]fluoranthene	367 (101%)	2580 (110%)	26400 (121%)
benzo[e]pyrene (4,5-benzopyrene)	443 (94%)	3100 (103%)	31600 (114%)
benzo[a]pyrene	330 (103%)	2310 (112%)	23600 (123%)
perylene	206 (90%)	1430 (98%)	14400 (110%)
3-methylcholanthrene	81.8 (57%)	505 (39%)	4340 (10%)
indeno[1,2,3-c,d]pyrene	253 (115%)	1790 (124%)	18500 (135%)
dibenz(a,h)anthracene	162 (109%)	1140 (118%)	11600 (130%)
benzo[g,h,i]perylene	188 (103%)	1320 (112%)	13500 (123%)

[11] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-28 250 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 15 days of exposure.

15 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[12]
naphthalene	29.3 (24%)	189 (13%)	1780 (13%)
benzo[b]thiophene	18.1 (25%)	110 (17%)	971 (6%)
2-methylnaphthalene	88.6 (38%)	562 (27%)	5180 (13%)
1-methylnaphthalene	82.5 (36%)	523 (25%)	4840 (10%)
biphenyl	120 (36%)	761 (24%)	7040 (10%)
1-ethylnaphthalene	210 (34%)	1340 (24%)	12400 (10%)
1,3-dimethylnaphthalene	263 (32%)	1680 (23%)	15600 (9%)
acenaphthylene	76.6 (38%)	486 (26%)	4480 (12%)
acenaphthene (1,8-hydroacenaphthylene)	147 (36%)	932 (25%)	8630 (10%)
4-methylbiphenyl	307 (28%)	1980 (21%)	18500 (11%)
2,3,5-trimethylnaphthalene	546 (23%)	3560 (22%)	33300 (16%)
fluorene (diphenylenemethane)	208 (33%)	1330 (23%)	12300 (8%)
1-methylfluorene	534 (24%)	3490 (24%)	32700 (18%)
dibenzothiophene	411 (26%)	2660 (21%)	24800 (11%)
phenanthrene	450 (25%)	2930 (22%)	27300 (14%)
anthracene	454 (24%)	2970 (24%)	27800 (19%)
2-methylphenanthrene	923 (28%)	6260 (40%)	59400 (43%)
9-methylanthracene	882 (27%)	5940 (38%)	56200 (39%)
3,6-dimethylphenanthrene	1570 (39%)	10900 (53%)	105000 (61%)
fluoranthene (1,2-benzacenaphthene)	984 (28%)	6680 (41%)	63800 (46%)
pyrene	1060 (32%)	7270 (45%)	69600 (51%)
2-methylfluoranthene	1580 (46%)	11100 (59%)	108000 (68%)
benzo[b]naphtho[2,1-d]thiophene	587 (53%)	4140 (65%)	40300 (74%)
benz[a]anthracene (1,2-benzanthracene)	611 (53%)	4300 (65%)	41900 (73%)
chrysene	420 (50%)	2950 (62%)	28600 (70%)
benzo[b]fluoranthene	624 (58%)	4430 (70%)	43300 (78%)
benzo[k]fluoranthene	324 (57%)	2290 (68%)	22300 (76%)
benzo[e]pyrene (4,5-benzopyrene)	434 (56%)	3080 (68%)	30100 (76%)
benzo[a]pyrene	271 (59%)	1920 (69%)	18500 (76%)
perylene	184 (57%)	1310 (68%)	12800 (76%)
3-methylcholanthrene	51.4 (48%)	355 (54%)	3200 (48%)
indeno[1,2,3-c,d]pyrene	165 (64%)	1180 (74%)	11400 (81%)
dibenz(a,h)anthracene	105 (62%)	749 (72%)	7270 (79%)
benzo[g,h,i]perylene	146 (59%)	1040 (70%)	10100 (79%)

[12] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-29 250 ppt Study: Average concentrations of PAHs accumulated in oysters and relative standard deviations (RSDs) as percent after 20 days of exposure.

20 days	(ng per g wet wt.)	(ng per g dry wt.)	(ng per g lipid)[13]
naphthalene	19.3 (27%)	142 (19%)	1440 (11%)
benzo[b]thiophene	13.3 (11%)	90.4 (8%)	877 (4%)
2-methylnaphthalene	65.6 (25%)	482 (17%)	4920 (9%)
1-methylnaphthalene	60.1 (25%)	442 (17%)	4510 (9%)
biphenyl	88.8 (25%)	653 (17%)	6660 (9%)
1-ethylnaphthalene	161 (24%)	1180 (17%)	12100 (9%)
1,3-dimethylnaphthalene	206 (25%)	1520 (16%)	15500 (8%)
acenaphthylene	54.7 (26%)	402 (17%)	4100 (9%)
acenaphthene (1,8-hydroacenaphthylene)	108 (25%)	797 (17%)	8130 (9%)
4-methylbiphenyl	269 (24%)	1980 (16%)	20200 (8%)
2,3,5-trimethylnaphthalene	506 (22%)	3730 (15%)	38200 (7%)
fluorene (diphenylenemethane)	164 (24%)	1210 (16%)	12300 (8%)
1-methylfluorene	503 (22%)	3720 (17%)	38000 (9%)
dibenzothiophene	356 (23%)	2630 (16%)	26800 (8%)
phenanthrene	406 (22%)	3000 (17%)	30600 (8%)
anthracene	460 (23%)	3410 (20%)	34800 (12%)
2-methylphenanthrene	1040 (20%)	7760 (21%)	79200 (15%)
9-methylanthracene	926 (22%)	6850 (19%)	70100 (13%)
3,6-dimethylphenanthrene	1960 (23%)	14600 (26%)	150000 (21%)
fluoranthene (1,2-benzacenaphthene)	1160 (20%)	8650 (21%)	88600 (15%)
pyrene	1300 (21%)	9710 (22%)	99200 (17%)
2-methylfluoranthene	2060 (26%)	15500 (30%)	158000 (25%)
benzo[b]naphtho[2,1-d]thiophene	865 (35%)	6500 (39%)	66100 (33%)
benz[a]anthracene (1,2-benzanthracene)	895 (34%)	6720 (39%)	68400 (32%)
chrysene	614 (32%)	4610 (37%)	47000 (30%)
benzo[b]fluoranthene	919 (42%)	6910 (47%)	70100 (40%)
benzo[k]fluoranthene	514 (43%)	3870 (48%)	39200 (40%)
benzo[e]pyrene (4,5-benzopyrene)	643 (42%)	4840 (47%)	49100 (40%)
benzo[a]pyrene	455 (50%)	3430 (56%)	34600 (47%)
perylene	303 (44%)	2280 (50%)	23100 (42%)
3-methylcholanthrene	63.6 (83%)	474 (93%)	4680 (83%)
indeno[1,2,3-c,d]pyrene	333 (54%)	2520 (61%)	25400 (52%)
dibenz(a,h)anthracene	203 (55%)	1540 (61%)	15500 (53%)
benzo[g,h,i]perylene	258 (46%)	1960 (53%)	19800 (45%)

[13] Note that it is inappropriate to normalize chemical concentration on a lipid basis unless steady state concentrations have been reached and this weighting reduces data variance These data are presented to show how normalization effects variance (as RSDs).

Table 3-30 250 ppt Study: Average PAH concentrations accumulated in SPMDs as ng/SPMD and relative standard deviation (RSD) as percent

Compounds	5 day	10 day	15 day	20 day
naphthalene	634 (3%)	803 (6%)	932 (2%)	994 (2%)
benzo[b]thiophene	379 (4%)	443 (9%)	516 (1%)	528 (2%)
2-methylnaphthalene	1140 (1%)	1660 (9%)	2260 (2%)	2710 (2%)
1-methylnaphthalene	1100 (1%)	1600 (9%)	2170 (2%)	2590 (2%)
biphenyl	1140 (1%)	1690 (9%)	2370 (2%)	2910 (2%)
1-ethylnaphthalene	1340 (3%)	2190 (7%)	3190 (4%)	4150 (2%)
1,3-dimethylnaphthalene	1440 (5%)	2440 (7%)	3600 (5%)	4810 (2%)
acenaphthylene	1100 (1%)	1560 (14%)	2150 (2%)	2580 (1%)
acenaphthene (1,8-hydroacenaphthylene)	1220 (2%)	1950 (7%)	2770 (4%)	3450 (2%)
4-methylbiphenyl	1390 (3%)	2360 (6%)	3520 (5%)	4700 (3%)
2,3,5-trimethylnaphthalene	1490 (7%)	2690 (7%)	4100 (8%)	5660 (3%)
fluorene (diphenylenemethane)	1290 (3%)	2100 (8%)	3140 (5%)	4040 (3%)
1-methylfluorene	1330 (7%)	2400 (10%)	3810 (9%)	5200 (4%)
dibenzothiophene	1340 (5%)	2270 (10%)	3490 (7%)	4620 (3%)
phenanthrene	1350 (6%)	2370 (9%)	3620 (8%)	4790 (4%)
anthracene	1240 (6%)	2230 (10%)	3540 (9%)	4860 (6%)
2-methylphenanthrene	1360 (10%)	2620 (10%)	4020 (12%)	5500 (5%)
9-methylanthracene	1380 (11%)	2520 (12%)	4220 (12%)	5710 (5%)
3,6-dimethylphenanthrene	1390 (13%)	2710 (10%)	4220 (13%)	5650 (5%)
fluoranthene (1,2-benzacenaphthene)	1210 (8%)	2250 (16%)	3620 (12%)	5030 (5%)
pyrene	1180 (8%)	2290 (14%)	3650 (12%)	5080 (5%)
2-methylfluoranthene	1240 (11%)	2480 (14%)	3830 (13%)	5440 (6%)
benzo[b]naphtho[2,1-d]thiophene	703 (18%)	1570 (11%)	2480 (13%)	3740 (13%)
1,2-benzanthracene	693 (18%)	1530 (13%)	2440 (14%)	3710 (12%)
chrysene	651 (20%)	1220 (15%)	1650 (12%)	2680 (12%)
benzo[b]fluoranthene (2,3-benzofluoranthene)	630 (22%)	1310 (15%)	1750 (13%)	2800 (11%)
benzo[k]fluoranthene (8,9-benzofluoranthene)	480 (23%)	965 (18%)	1240 (12%)	2200 (14%)
benzo[e]pyrene	434 (20%)	1100 (18%)	1590 (11%)	2570 (12%)
benzo[a]pyrene	414 (23%)	974 (23%)	1360 (12%)	2360 (13%)
perylene	293 (19%)	969 (19%)	1380 (15%)	2470 (16%)
3-methylcholanthrene	295 (23%)	1060 (24%)	1520 (17%)	2830 (16%)
indeno[1,2,3-c,d]pyrene	349 (23%)	1040 (22%)	1380 (16%)	2530 (16%)
dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)	194 (23%)	847 (26%)	1030 (17%)	2140 (18%)
benzo[g,h,i]perylene (1,12-benzoperylene)	253 (21%)	882 (25%)	1170 (16%)	2270 (17%)

DILUTER WATER SAMPLE RESULTS

Measured diluter performance indicates very consistent spike delivery in all exposures. The 10 ppt exposure concentration for naphthalene (15.4 ng/L) slightly exceeded the expected level due to naphthalene originating in the PVC plumbing of the laboratory or other background sources. Similar sources are responsible for the slight increase in methyl-naphthalenes. Measured water concentrations are within 30% of the nominal concentrations for all studies for naphthalene though the mid-molecular weight PAHs anthracene and methyl-phenanthrene and measured reproducibilities are good (with some exceptions in the 10 ppt study). Difficulties in the water sampling methodology are in part responsible for reproducibilities beyond methyl-fluoranthene approaching and exceeding 20%. Grab sampling water and liquid-liquid extraction is difficult with samples like seawater that are high in dissolved organic carbon (DOC). PAHs beyond benzo[b]naphtho[2,1-d]thiophene are consistently measured to be 55 to 35% of the nominal values in the 100 ppt and 250 ppt studies and 35 to 25% of the nominal 10 ppt level (Table 3-31). Possible explanations for these lower concentrations include sorption to surfaces, particulates and DOC; uptake by the bivalves and SPMDs; in situ degradation processes (e.g., biodegradation, photo-oxidation) and others. The diluter delivery system is very homogeneous, constructed of very inert and low sorption materials (glass, Teflon® and Kynar® - a polyfluorinated polymer like Teflon) and essentially completely closed so system losses are not expected to be responsible. Considering the maximum uptake rates of both the SPMDs and oysters at most suggests a maximum lowering of the concentrations of 20% so sequestering may not be the cause (assuming PAHs are readily not biotransformed by the bivalves so sequestered amounts reflect the only source of removal). Biological growth did take place but was kept minimal and would not have created such a consistent ratio among the studies. Sorption to the shell exteriors is certainly a possibility; the surface is highly porous and covered, despite scrubbing, with biological materials rich in organic carbon. However, because of the consistent ratios and the (expected) consistent levels of DOC in the ambient sea water, sorption to DOC is a likely the reason for the lower measured levels. It must be emphasized that delivery was very uniform in all studies and that diluter design insured that SPMDs and oysters were exposed to equivalent concentrations in every exposure, at all times. Selection of nominal or measured (extracted sample) values as the most representative of the actual water concentrations is problematic.

Table 3-31 Average PAH water concentrations as a fraction of the nominal study value

Compound	250 ppt	100 ppt	10 ppt
naphthalene	0.92	1.00	1.54
benzo[b]thiophene	0.92	0.95	0.95
2-methylnaphthalene	0.87	0.95	1.14
1-methylnaphthalene	0.89	0.94	1.08
biphenyl	0.86	0.89	0.87
1-ethylnaphthalene	0.84	0.87	0.82
1,3-dimethylnaphthalene	0.86	0.80	0.88
acenaphthylene	0.92	0.85	0.91
acenaphthene (1,8-hydroacenaphthylene)	0.88	0.82	0.87
4-methylbiphenyl	0.86	0.76	0.74
2,3,5-trimethylnaphthalene	0.88	0.85	0.95
fluorene	0.85	0.92	1.03
1-methylfluorene	0.85	0.91	0.94
dibenzothiophene	0.81	0.88	0.86
phenanthrene	0.80	0.87	0.92
anthracene	0.74	0.76	0.69
2-methylphenanthrene	0.78	0.80	0.78
9-methylanthracene	0.61	0.67	0.64
3,6-dimethylphenanthrene	0.76	0.77	0.72
fluoranthene (1,2-benzacenaphthene)	0.72	0.75	0.67
pyrene	0.72	0.74	0.66
2-methylfluoranthene	0.72	0.71	0.63
benzo[b]naphtho[2,1-d]thiophene	0.54	0.52	0.34
benz[a]anthracene (1,2-benzanthracene)	0.53	0.48	0.32
chrysene	0.59	0.57	0.38
benzo[b]fluoranthene (2,3-benzofluoranthene)	0.53	0.44	0.31
benzo[k]fluoranthene (8,9-benzofluoranthene)	0.53	0.43	0.30
benzo[e]pyrene(4,5-benzopyrene)	0.53	0.45	0.35
benzo[a]pyrene	0.47	0.38	0.25
perylene	0.46	0.42	0.27
3-methylcholanthrene	0.13	0.20	0.14
indeno[1,2,3-c,d]pyrene	0.44	0.31	0.22
dibenz[a,h]anthracene (1,2:5,6-dibenzanthracene)	0.41	0.28	0.18
benzo[g,h,i]perylene (1,12-benzoperylene)	0.44	0.35	0.26

PAH TOTALS IN OYSTERS AND SPMDS

On average and for any exposure time, oysters accumulated about 5 times less per gram of wet weight than the half HUCK SPMD. On a dry weight basis, oyster totals were about 40% higher than a half HUCK SPMD while lipid weighting the oysters results suggests an average of 14 times higher total PAHs. Scaling to an average oyster and a full one HUCK SPMD, as would be used in the field, indicates one SPMD is about equivalent to one oyster in terms of total PAHs sequestered for equal exposures noting that these estimates are appropriate only for uptake from dissolved phase and over the 20 day period. Eventually the higher capacity of the SPMD is expected to sequester more analyte than an oyster; the effective lipid content of the SPMD is greater than 1 gram while oysters contain approximately 250 milligrams of lipid.

Total PAH Oyster Accumulation with Time

Although there is a great deal of dispersion in the oyster data, the plots of the total amounts normalized to the amount accumulated at day 20 show the expected curvilinear behavior (Figure 3-1, Figure 3-3, and Figure 3-5). The 250 ppt exposure shows the most ideal uptake curve for the oysters (Figure 3-5). The largest percentage of the total is accumulated in the first 5 days (roughly 35% - 40% in the 100 and 250 ppt studies) with a nearly linear uptake after that. In general, data trends on a wet, dry and lipid weight basis agree with the exception of the 15 day exposed oysters of the 100 ppt study. The apparent discontinuities in curves for the day 15 values in the 10 ppt study and day 10 in the 100 ppt study must be compared to the uncertainties (Table 3-4 and Table 3-14) which exceed ±20%. Even though the oysters were selected to be uniform in size, their filtration, ventilation, sequestering behavior, etc., are not likely to be uniform and the "ideal" curve of the 250 ppt study is likely fortuitous.

Oyster and SPMD data : basis of comparison

Besides the obvious problem of completely different matrices, oyster data may be presented and interpreted on wet, dry and lipid weight bases. Typically, data is cited on a dry weight basis (e.g., Mussel Watch data). In terms of the average total PAHs accumulated in oyster tissue there is a

great deal of dispersion in the data regardless of the basis, however, the dry weight basis seems most suitable.

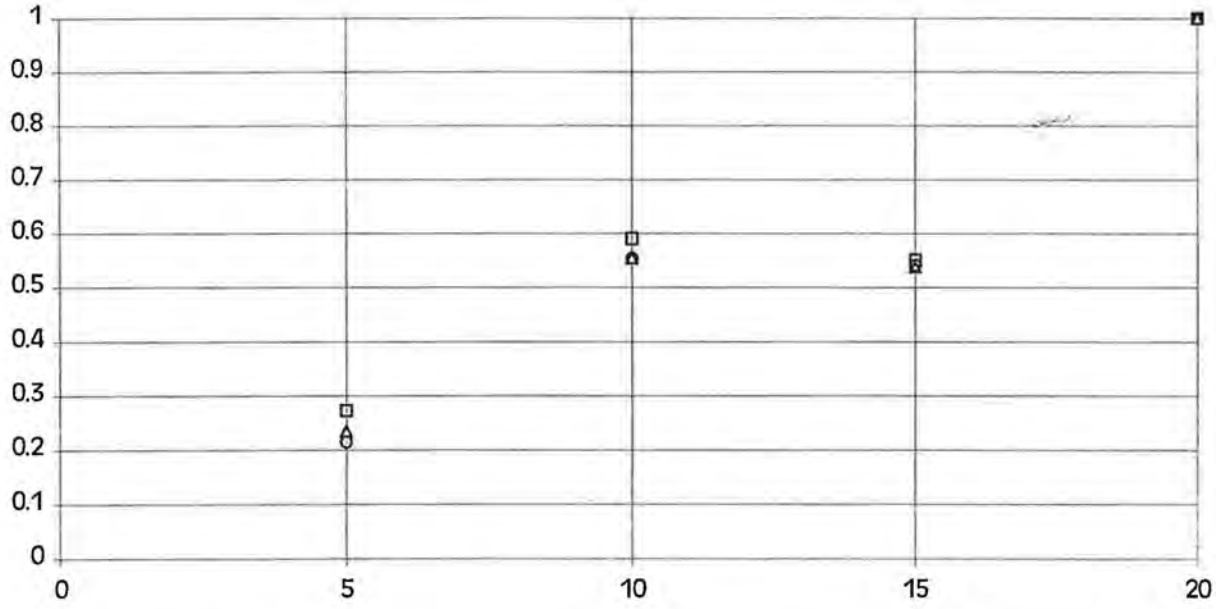

Figure 3-1 10 ppt Study: Oyster PAH totals normalized to amount accumulated at day 20: squares, wet weight basis; triangles, dry weight basis; circles, lipid weight basis

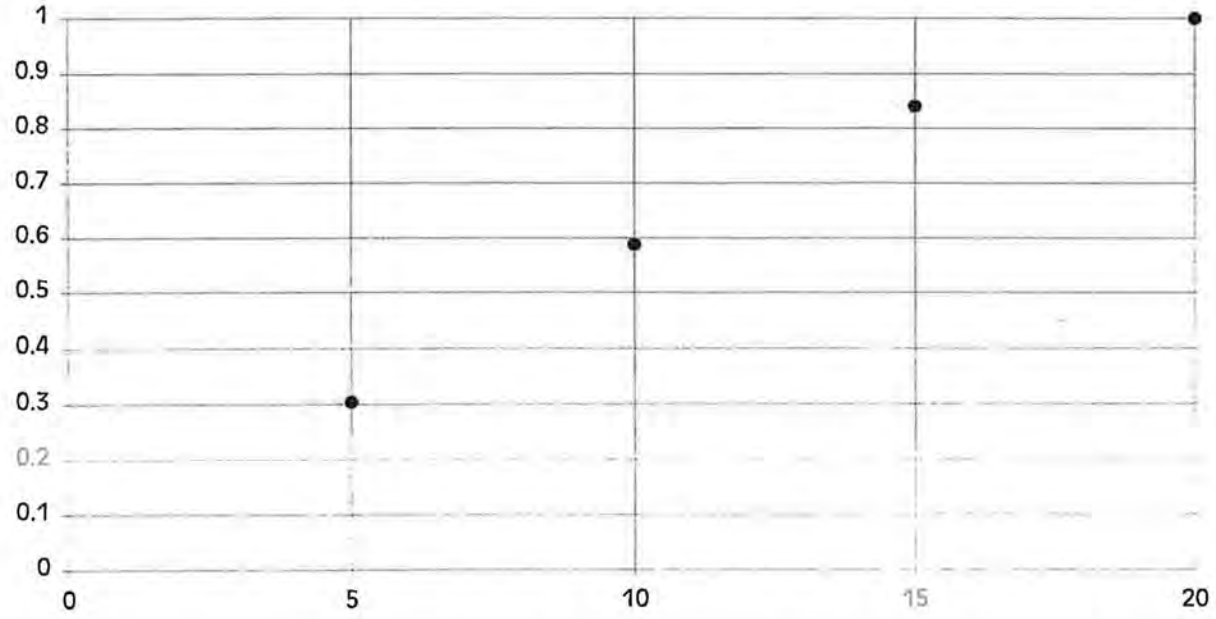

Figure 3-2 10 ppt Study: SPMD PAH totals normalized to amount accumulated at day 20

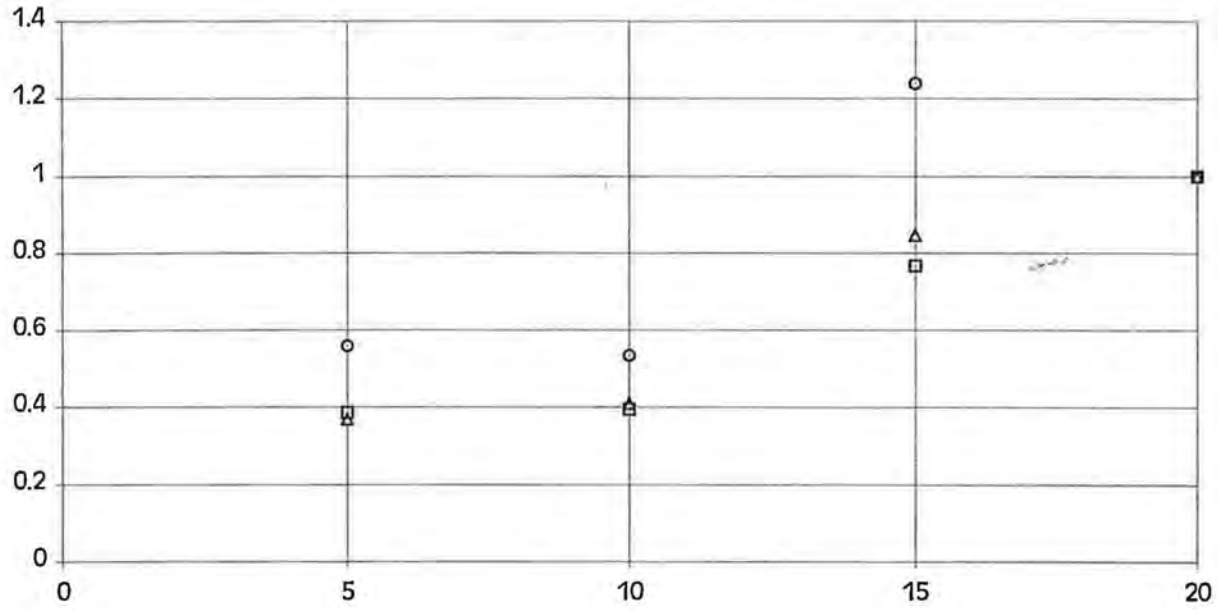

Figure 3-3 100 ppt Study: Oyster PAH totals normalized to amount accumulated at day 20: squares, wet weight basis; triangles, dry weight basis; circles, lipid weight basis

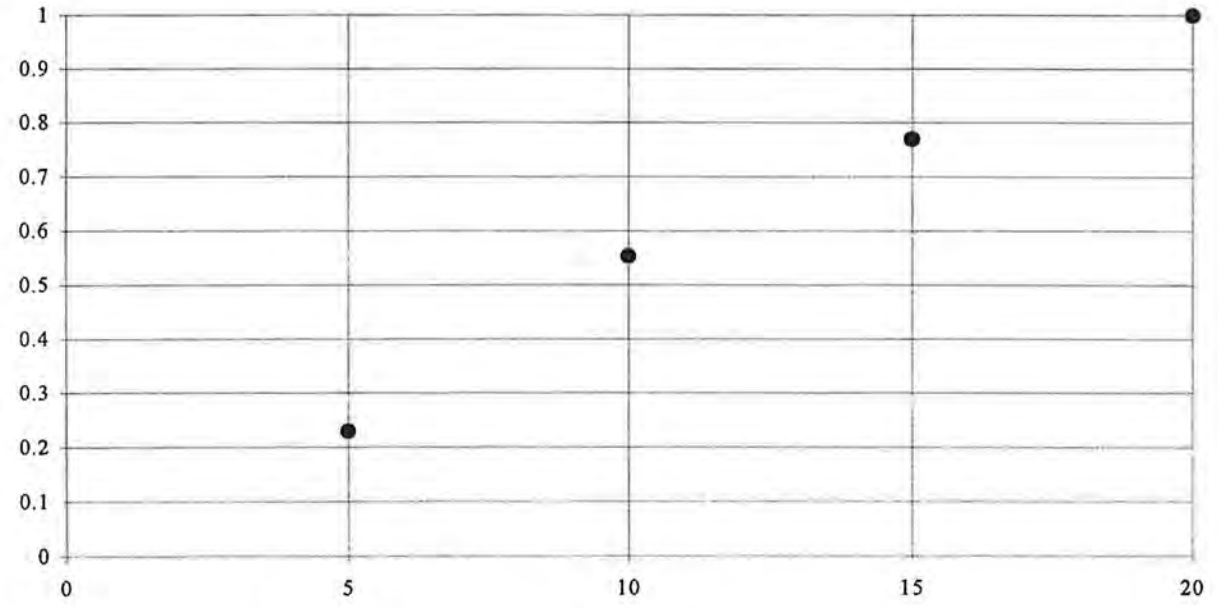

Figure 3-4 100 ppt Study: SPMD PAH totals normalized to amount accumulated at day 20

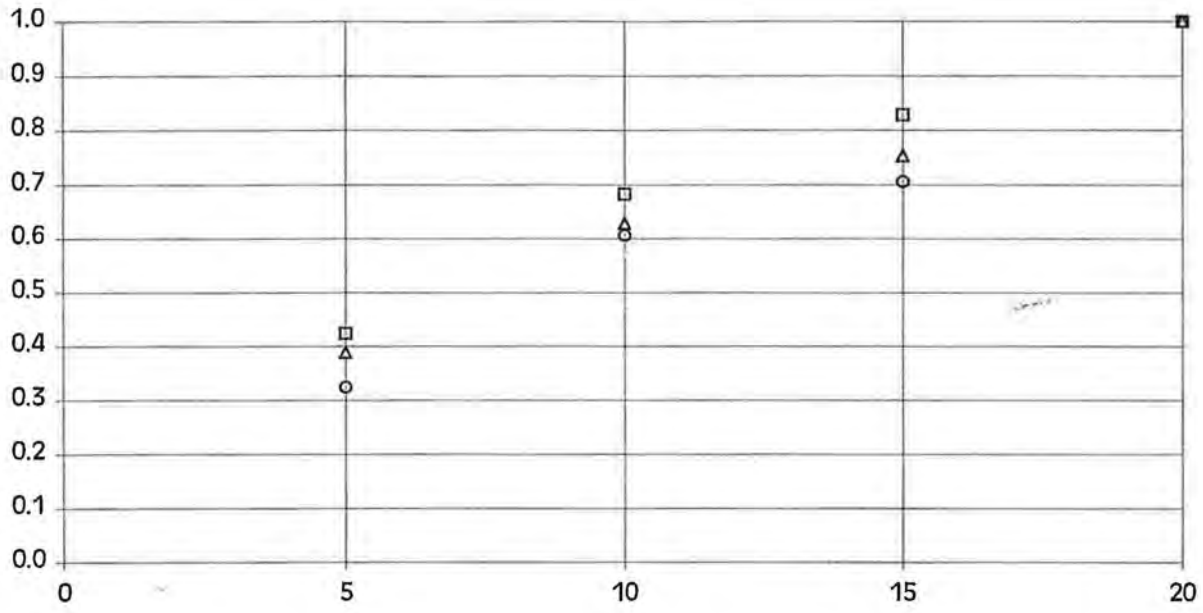

Figure 3-5 250 ppt Study: Oyster PAH totals normalized to amount accumulated at day 20: squares, wet weight basis; triangles, dry weight basis; circles, lipid weight basis

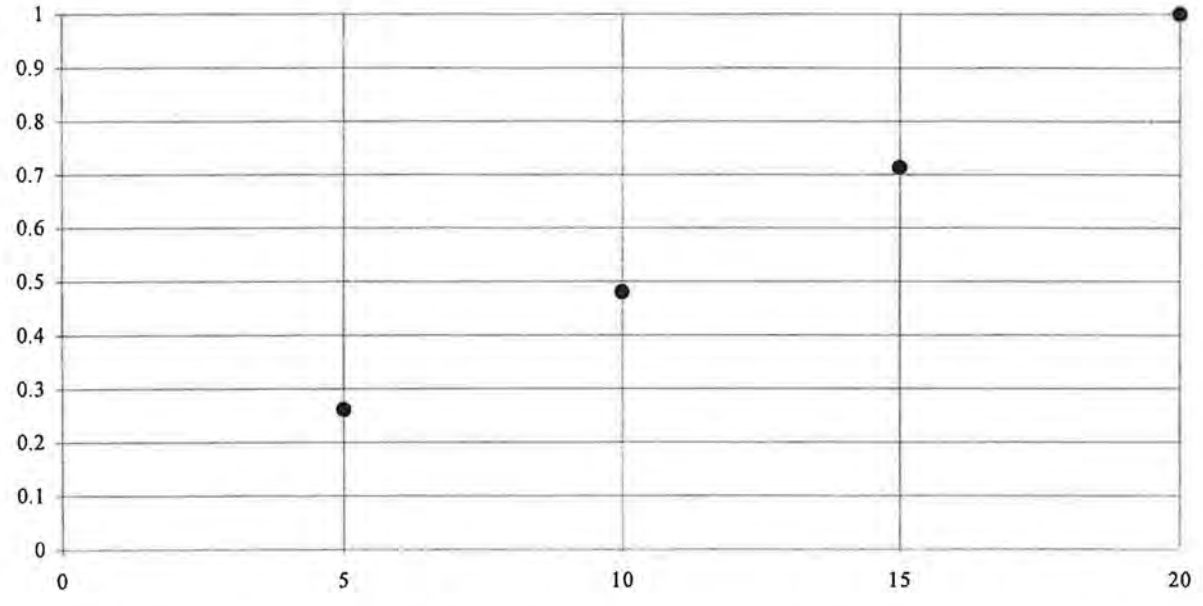

Figure 3-6 250 ppt Study: SPMD PAH totals normalized to amount accumulated at day 20

Total PAH Accumulation in SPMDs with Time

In contrast to the oysters, total amounts of PAHs sequestered by SPMDs show very high reproducibilities agreeing to within 6% on average (Table 3-5, Table 3-15, and Table 3-25). The normalized curves are nearly identical in all three studies and the curves themselves are nearly linear; 25% of the 20 day total in 5 days, about 50% in 10, etc. (Figure 3-2, Figure 3-4, and Figure 3-6). The curves for total PAHs in 250 ppt and 100 ppt exposures look very similar and nearly linear but the 10 ppt study is slightly higher although perhaps not significantly.

INDIVIDUAL PAH ACCUMULATION

Appendix A provides graphs of the accumulation of the individual PAHs in oysters and SPMDs with exposure time for all three studies. Oyster data is given on a dry weight basis and the result for each oyster is cited. Similarly, PAHs in each SPMD are given. The relatively high individual variation is again apparent in the oyster results. In contrast, the SPMDs results were very reproducibility.

Oysters

There is a great deal of similarity in the overall PAH profiles in the oysters for the various exposure times and studies (Figure 3-7, Figure 3-9, and Figure 3-11). The first 10 compounds and compound #12 reach steady state rapidly and have the lowest concentration factors: i.e., (1), naphthalene; (2), benzo[b]thiophene; (3), 2-methylnaphthalene; (4), 1-methylnaphthalene; (5), biphenyl; (6), 1-ethylnaphthalene; (7), 1,3-dimethylnaphthalene; (8), acenaphthylene; (9), acenaphthene; (10), 4-methylbiphenyl; (12), fluorene. An approximately constant and equivalent concentration factor is found in the next series of compounds: i.e., (11), 2,3,5-trimethylnaphthalene; (13), 1-methylfluorene; (14), dibenzothiophene; (15), phenanthrene; (16), anthracene. An increase sequestration for (17), 2-methylphenanthrene and (18), 9-methylanthracene, with an interesting "preference" for 2-methylphenanthrene over 9-methylanthracene which appears in all studies, is mirrored in the next two PAHs, (19), 3,6-dimethylphenanthrene and (20), fluoranthene. However, the high degree of variance in the oyster data makes definitive statements about potential differences difficult. In the 100 ppt and 250 ppt

studies 2-methylfluoranthene, (22), and 3,6-dimethylphenanthrene, (19), are the most prevalent compounds at all times. Higher molecular weight PAHs beyond dimethyl-phenanthrene, (22), show a drop to more or less equivalent accumulation in (23), benzo[b]naphtho[2,1-d]thiophene, and (24), benz[a]anthracene, followed by a steady decline from chrysene, (25), through benzo[b]fluoranthene, (26), benzo[k]fluoranthene, (27), benzo[e]pyrene, (28), benzo[a]pyrene, (29), to perylene (30). Compounds 31 to 34 show essentially equivalent accumulation: (31), 3-methylcholanthrene; (32), indeno[1,2,3-c,d]pyrene; (33), dibenz[a,h]anthracene; (34), benzo[g,h,i]perylene. While this pattern appears in the 100 ppt and 250 ppt studies, benzo[e]pyrene, (28), benzo[b]naphtho[2,1-d]thiophene, (23), benzo[b]fluoranthene, (26), are most prevalent in the 10 ppt exposure profile. Still the pattern in the compounds up to 3,6-dimethylphenanthrene, (19), also appears in the 10 ppt study and only the higher molecular weight compounds 22 and beyond contribute a greater portion to the profile than in the other two studies. A concentration dependence of the bioconcentration of certain PAHs is implied.

SPMDs

The patterns are similar in all SPMDs for all studies (Figure 3-8, Figure 3-10, and Figure 3-12). The least accumulation at any time is for (2), benzo[b]thiophene, essentially non-detectable in the 10 ppt study, followed by naphthalene. While the methyl-naphthalenes show similar sequestration, (3), 2-methylnaphthalene and (4), 1-methylnaphthalene, compounds 5,6, and 7 show increasing retention a trend also apparent in the oysters; biphenyl, 1-ethylnaphthalene, and 1,3-dimethylnaphthalene respectively. The trend in increasing sequestration drops for acenaphthylene, (8), in both SPMDs and in oysters. The mid-range PAHs between 10 and 22 show similar sequestering and contribute much more to the profile than the comparable compounds in the oysters. Compounds higher than 22 show a declining contribution to the profile, very similar to that observed in the oysters.

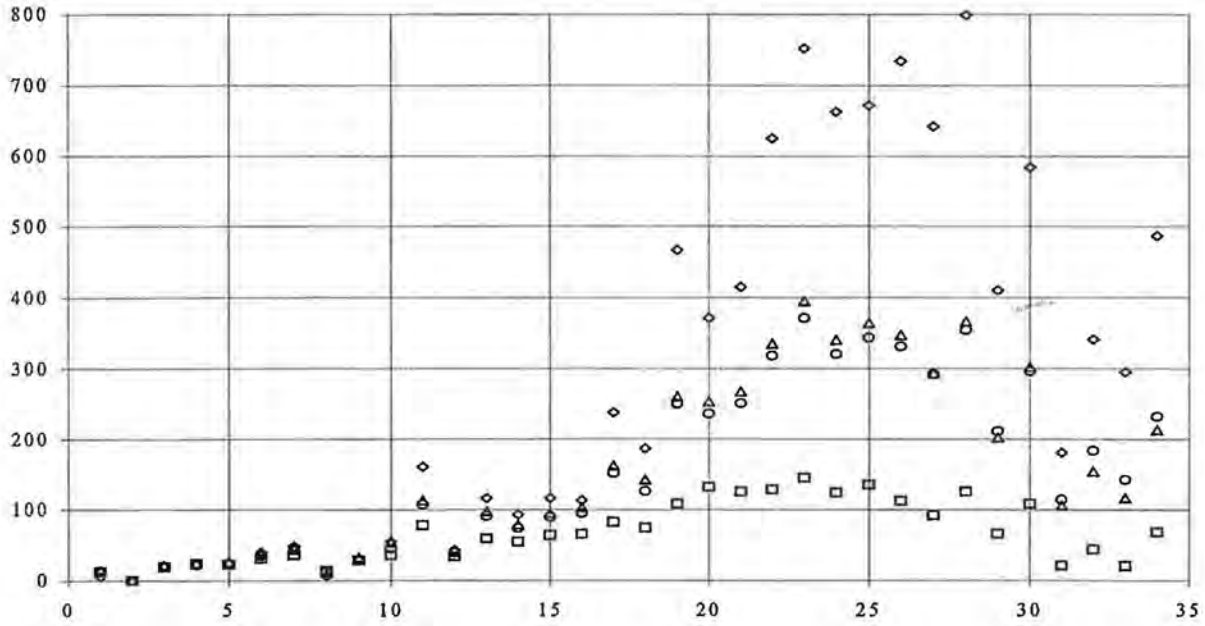

Figure 3-7 10 ppt Exposure: PAH concentrations in oysters as ng/ g dry weight versus compound index (see text for identifying numbers); 5 day, squares; 10 day, triangles; 15 day, circles; and diamonds, 20 day.

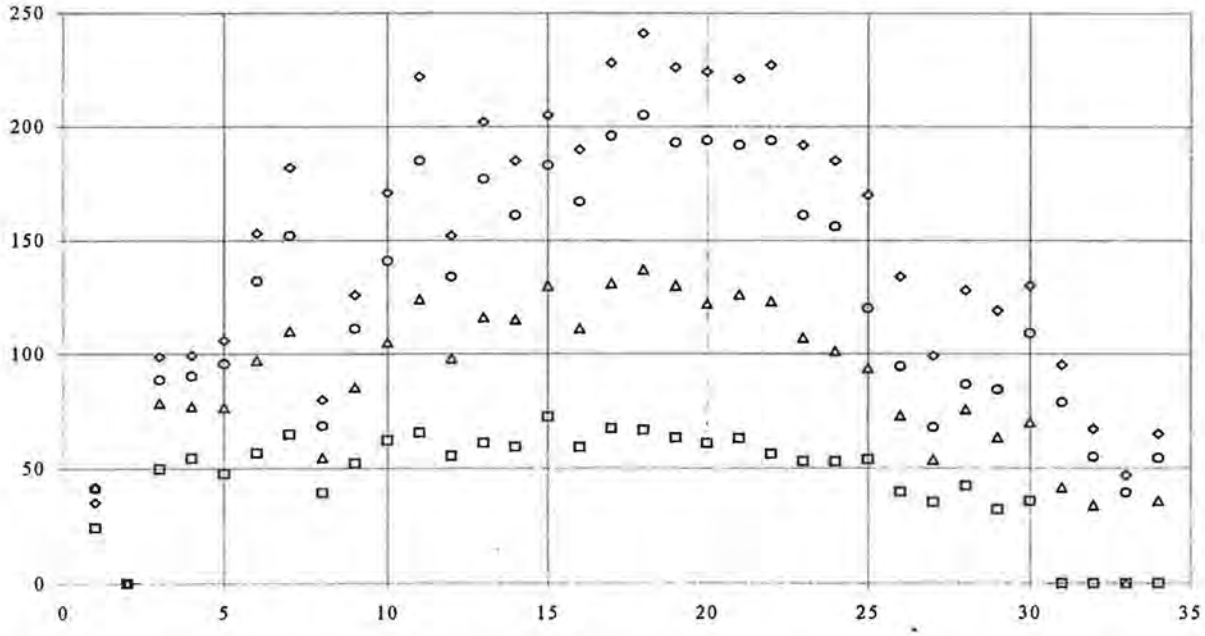

Figure 3-8 10 ppt Exposure: PAH concentrations in SPMDs as ng/SPMD versus compound index (see text for identifying numbers); 5 day, squares; 10 day, triangles; 15 day, circles; and diamonds, 20 day.

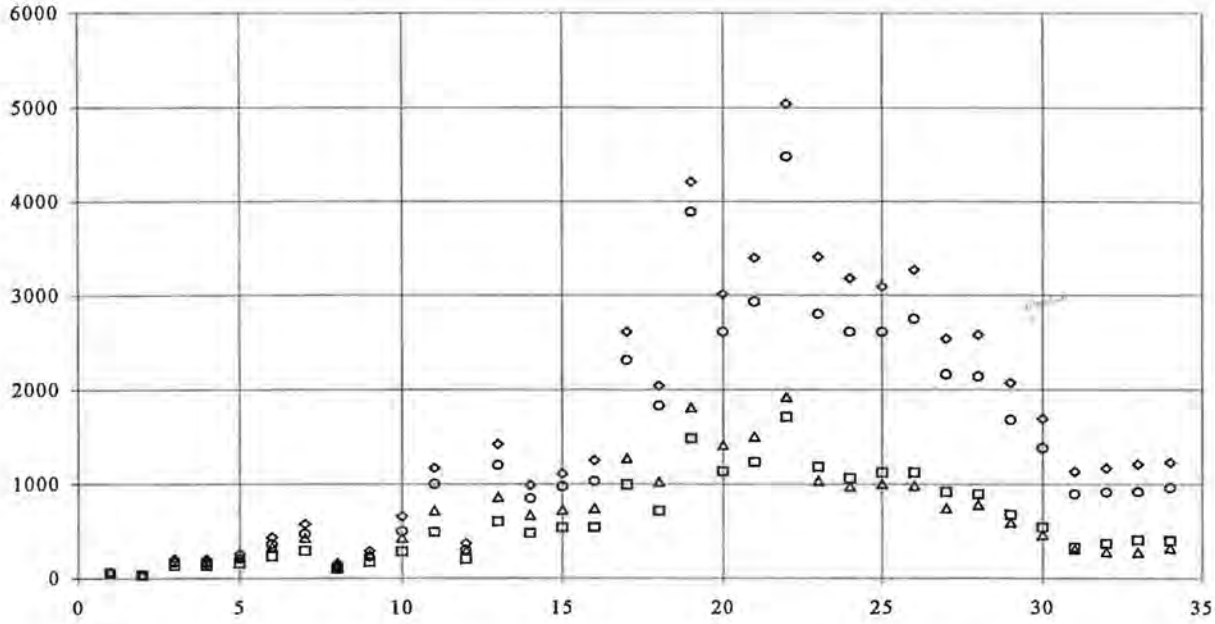

Figure 3-9 100 ppt Exposure: PAH concentrations in oysters as ng/ g dry weight versus compound index (see text for identifying numbers); 5 day, squares; 10 day, triangles; 15 day, circles; and diamonds, 20 day.

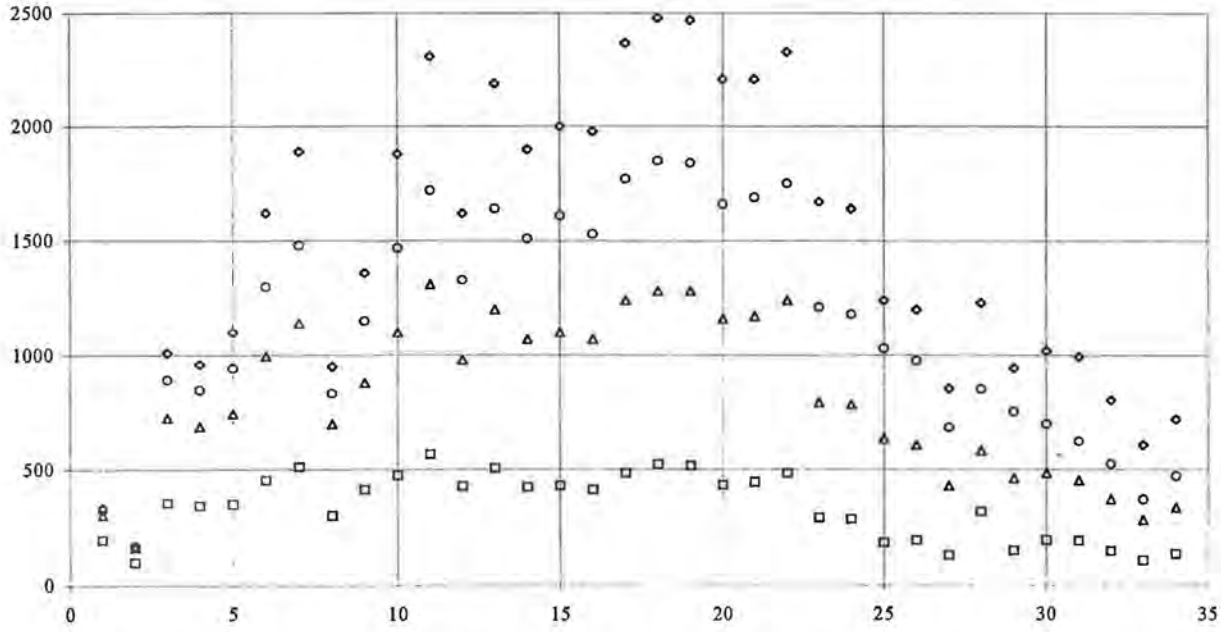

Figure 3-10 100 ppt Exposure: PAH concentrations in SPMDs as ng/SPMD versus compound index; 5 day, squares (see text for identifying numbers); 10 day, triangles; 15 day, circles; and diamonds, 20 day.

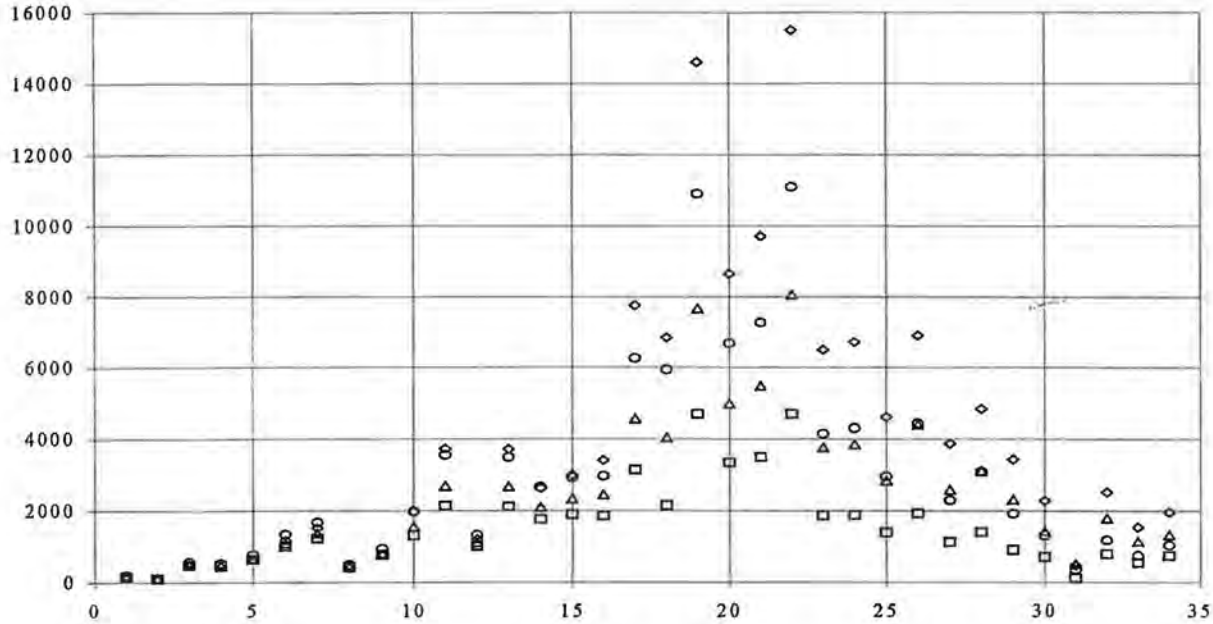

Figure 3-11 250 ppt Exposure: PAH concentrations in oysters as ng/ g dry weight versus compound index (see text for identifying numbers); 5 day, squares; 10 day, triangles; 15 day, circles; and diamonds, 20 day.

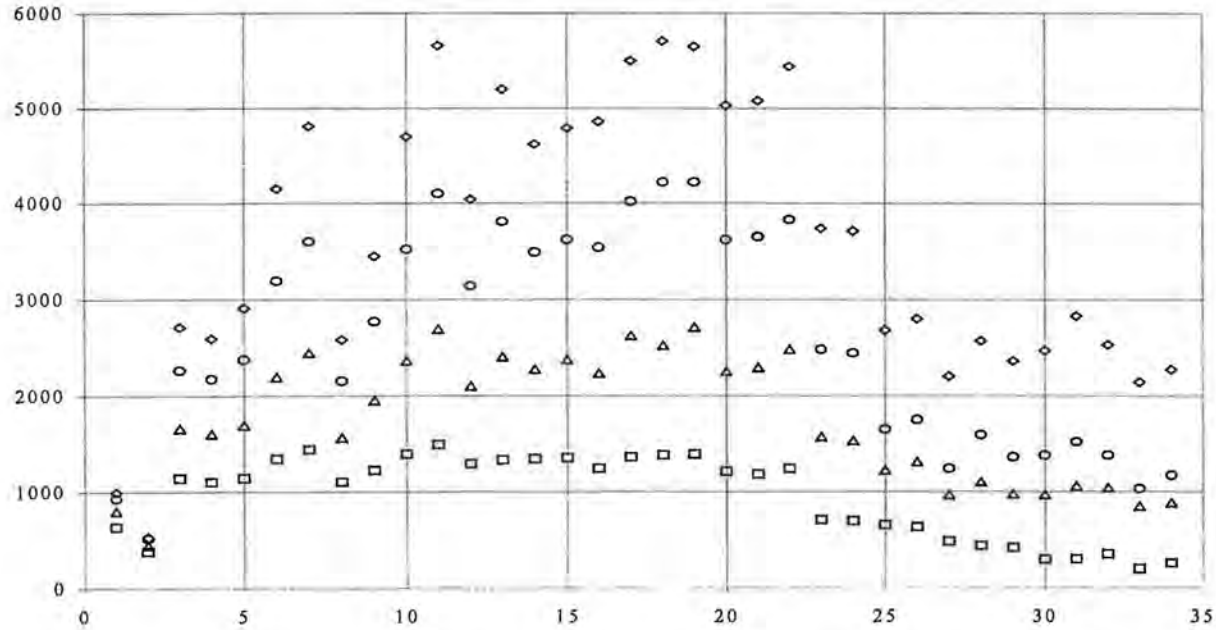

Figure 3-12 250 ppt Exposure: PAH concentrations in SPMDs as ng/SPMD versus compound index (see text for identifying numbers); 5 day, squares; 10 day, triangles; 15 day, circles; and diamonds, 20 day.

Kinetic Considerations

Uptake kinetics are visible in the figures, the most salient feature being the incremental changes in the amount in the SPMDs. Appendix B provides a better method of viewing the kinetics of all the studies as ratios of the amount at each day to the amount at 20 days. These ratios also provide a simple way of distinguishing the uptake or sampling kinetics regime and the approach to steady state which is the topic of the next section.

Chapter 4
DISCUSSION

In this section the kinetic aspect of the data will considered. Data will be cited on the basis of oyster dry weight and the half HUCK SPMD design used in the experiments; the values can be scaled to other bases as appropriate. The objective is to compare and mathematically characterize the kinetic behavior of the uptake of PAHs by oysters and SPMDs and draw conclusions concerning their use as environmental monitors of aquatic systems.

KINETICS AND RATIO PLOTS

Appendix B presents the oyster and SPMD accumulation of the individual PAHs for all exposures as the ratio (R) of the average concentration at day 5, 10 or 15 to that measured at day 20. Essentially the average concentrations are normalized to that of the last day of the exposure and plotted against time. This representation has the advantages of allowing dry and lipid weighted oyster data for all three studies to be presented on the same graph so anomalies due to lipid weighting are easily recognized; trends in sampling kinetics are readily apparent; and in some cases, the clearance rate constant k_2 can be estimated from the plots. Concentration factors (CFs) can be calculated by multiplying the ratio for any day by the concentration factor for day 20; i.e., concentration at day 20 divided by the water concentration.

OYSTER PAH TRENDS

The relationship of the compound's K_{ow} to its uptake is as expected; uptake kinetics become increasingly linear as K_{ow} increases. For example, as the number of benzenoid rings increase in the series naphthalene, phenanthrene and chrysene, the ratio plot shows a more gradual approach to steady state (Figure 4-1). Naphthalene reaches steady state in less than 5 days, phenanthrene uptake shows curvilinear behavior, and chrysene exhibits essentially linear uptake. The alkylated naphthalene series shows the same pattern of increasing linearity or decreasing curvilinearity with increasing K_{ow} , however k_2 is large enough to be reflected in the data (Figure 4-2).

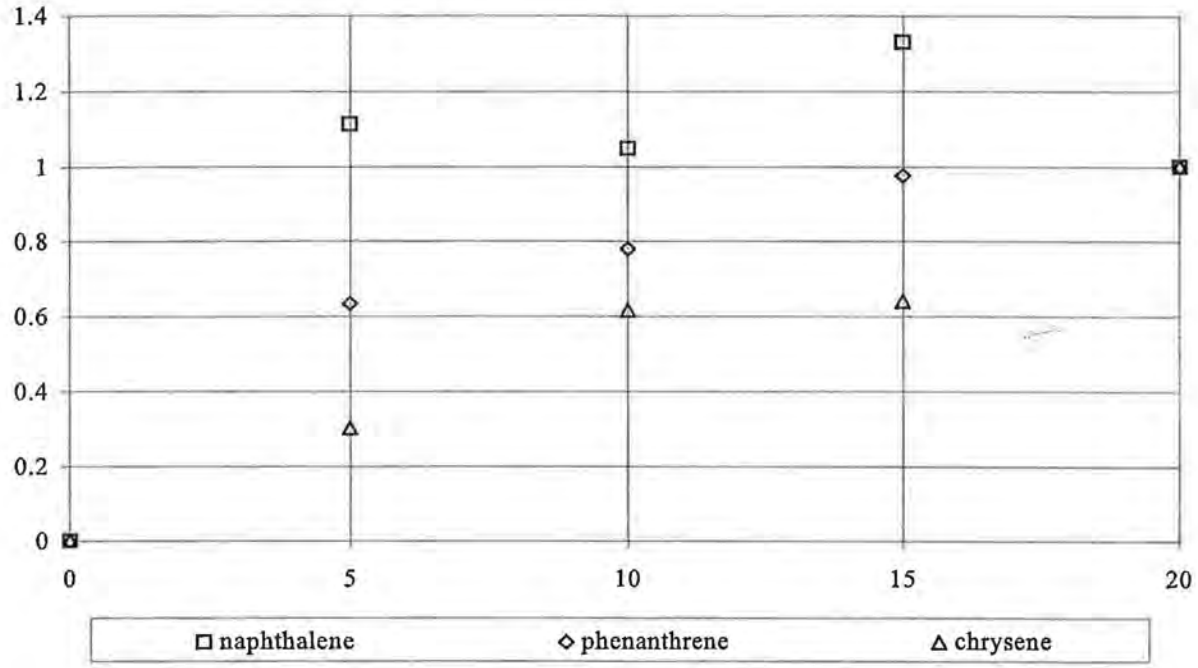

Figure 4-1 250 ppt Study: average 2, 3, and 4 ring PAH dry weight oyster concentrations normalized to the average amount at day 20 versus exposure period in days

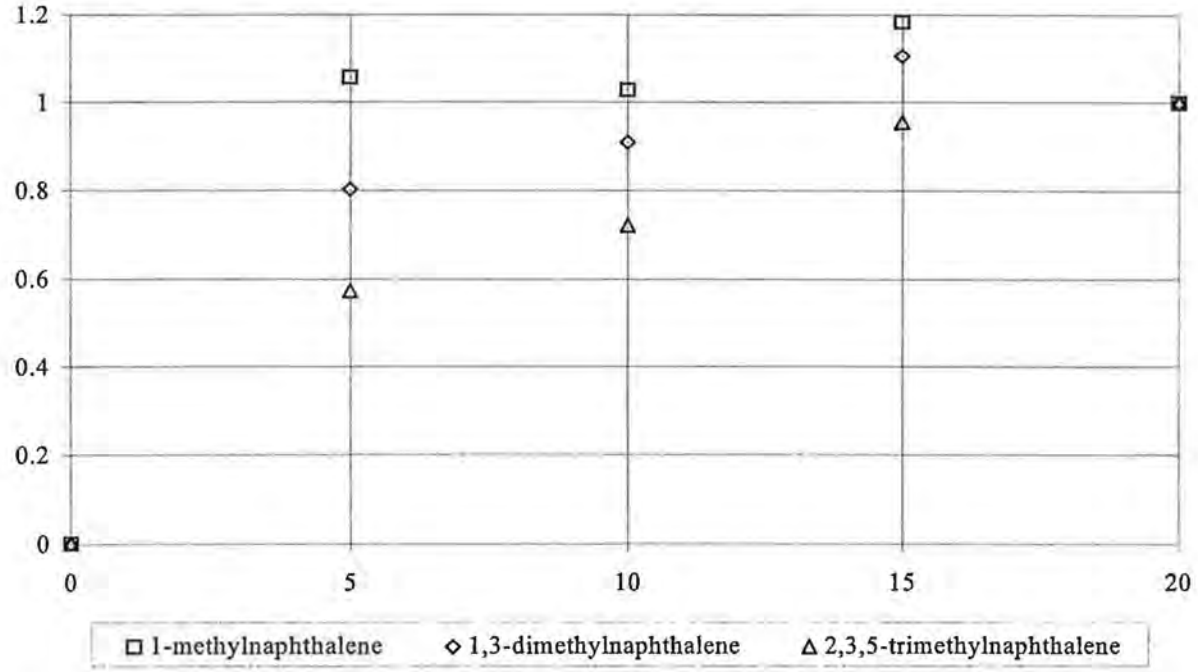

Figure 4-2 250 ppt Study: average alkylated naphthalene dry weight oyster concentrations normalized to the average amount at day 20 versus exposure period in days

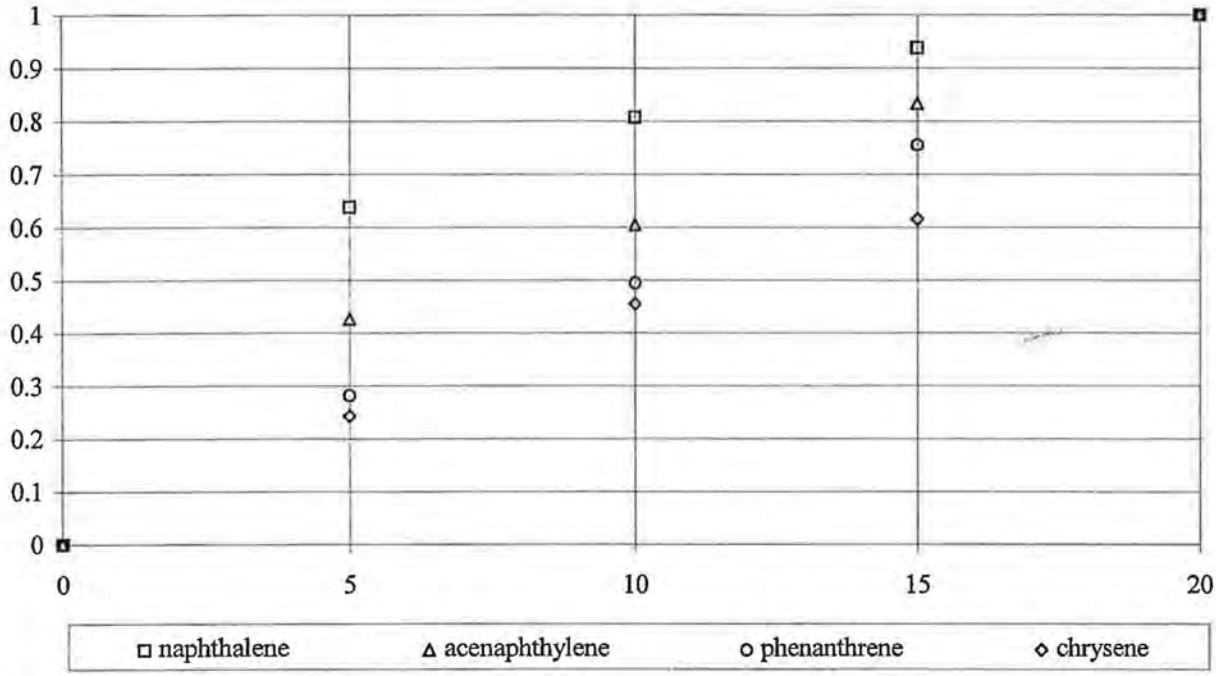

Figure 4-3 250 ppt Study: average 2, 3, and 4 ring PAH SPMD concentrations normalized to the average amount at day 20 versus exposure period in days

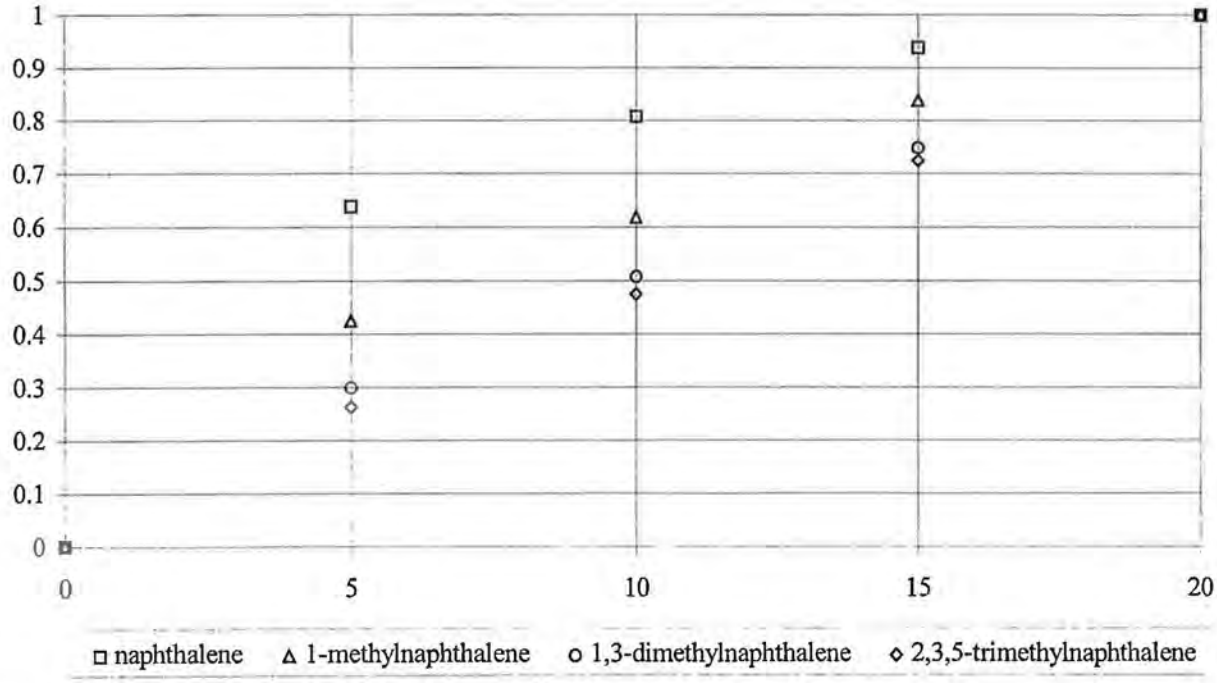

Figure 4-4 250 ppt Study: Normalized alkylated naphthalene PAH SPMD concentrations normalized to the average amount at day 20 versus exposure period in days

SPMD PAH TRENDS

Similarly, the kinetics of SPMD accumulation of the PAHs also shows an increasing trend toward linearity as K_{ow} increases. As the number of rings increases from 2 to 4 (Figure 4-3) or the degree of alkylation of increases (Figure 4-4), the ratio plots show a decreasing clearance rate or smaller value of k_2 (see following section on modeling) and consequently a slower approach to steady state.

Comparing the kinetics of naphthalene in oysters to that in SPMDs shows that steady state is reached in less than 5 days for the oysters while a slower, more curvilinear approach to steady state is demonstrated by the SPMDs. As can be seen in the ratio plots in Appendix B, this is true for all compounds; **steady state is more rapidly reached by oysters than by SPMDs for all PAHs**.

INTERPRETATION OF RATIO PLOT DATA - ESTIMATES OF k_2

The power of ratio plots is that they are independent of the basis of comparison and directly reveal the influence of the clearance rate constant on the data. A more detailed development of the single compartment model and the mathematical relations used in the following discussion is presented in the Mathematical Appendix, Appendix M[1]. SPMDs and oyster sequestration data can be described by a single compartment model. The model is based on the assumption that the analyte concentration in the environmental monitor at time t, C_t, is equal to the analyte concentration at steady state, Css , modified by an exponential term dependent on the clearance or dissipation rate, k_2 ;

$$C_t = C_{ss}\left(1 - e^{-k_2 t}\right)$$

(Equation 4-1)

[1] Reading this appendix may make the following discussion clearer. The bottom line is, do not give up because of the math.

Notice k_2 has units of inverse time, e.g., day^{-1}. If the product k_2 t is very small, then the concentration at t is given by

$$C_t = C_{ss} k_2 t = k_1 W t$$

(Equation 4-2)

where k_1 is the uptake rate and W is the water concentration. (Note that Css can be expressed as the product of the steady state bioconcentration factor and the water concentration for oysters, Css = BCF•W and the product of the equilibrium partition coefficient and the water concentration for the SPMDs, Css = K •W , because BCF ≡ Css / W = k_1/k_2 and K = k_1/k_2.) This (Equation 4-2) implies there must be a linear region of uptake where concentration changes are directly proportional to the water concentration and exposure time. The duration of this period is determined by the magnitude of k_2 • t.

The ratio plots are based on the definition of R_t as the ratio of the concentration at t to that at day 20, C_t / C_{20}, so

$$R_t \equiv \left(\frac{C_t}{C_{20}}\right) = \frac{\left(1 - e^{-k_2 t}\right)}{\left(1 - e^{-k_2 (20 days)}\right)}$$

(Equation 4-3)

This ratio is independent of Css, dependent only on time and k_2, and therefore independent of whether the data is being discussed on a wet, dry, lipid weight basis or whether it is oyster data or SPMD data. If k_2 is very small then, using (Equation 4-2),

$$R_t \equiv \left(\frac{C_t}{C_{20}}\right) \xrightarrow{k_2 t \to 0} \frac{t}{20 days}$$

(Equation 4-4)

Therefore the Ratio Plots reveal linear behavior as the approach to the fractional exposure times; i.e., if linear uptake is occurring then at 5 days $R_5 = .25$, $R_{10} = 0.5$, $R_{15} = 0.75$. However, if there is a substantial influence of k_2 and (the uptake line is curved, not linear) then the analysis presented in the Mathematical Appendix shows that the ratios can be used to estimate k_2 -

$$\frac{C_{t'} - C_t}{C_5} = \frac{R_{t'} - R_t}{R_5} = e^{-k_2 t} \Rightarrow \ln\left(\frac{R_{t'} - R_t}{R_5}\right) = -k_2 t$$

(Equation 4-5)

where there are two possible cases; t'=20 days and t = 15 days or t'=15 days and t = 10 days. (More rigorous determinations of k_2 are possible by nonlinear regression of the uptake data and directly from depuration experiments as described later.) The essential point is that the ratio plots directly reveal the effect of the clearance constant, k_2 , as the degree of deviation from linearity in uptake data.

WHY IS k_2 IMPORTANT?

Rewriting an earlier equation (Equation 4-1) reveals the role of k_2 -

$$\frac{C_t}{C_{ss}} = 1 - e^{-k_2 t}$$

(Equation 4-6)

Simply stated, k_2 controls the rate of approach to steady state. For example, the time to reach half saturation (or equivalently the time to depurate half the amount present, the half-life) is

$$- \ln (0.5) = 0.639 = k_2 \, t_{½} \quad \text{or} \quad \tau_{½} = 0.693 / k_2$$

Therefore, environmental monitors with smaller clearance rates (k_2s) have longer retention times for compounds and allow event integration over greater periods. In other words, a transient event or "spill" has a greater chance of being detected if the monitor has a lower k_2. The magnitude of the steady state concentration is controlled by the ratio of k_1 to k_2 so the ultimate capacity of the device is given by the ratio.

As an example, in this study naphthalene reaches a concentration in oysters experimentally indistinguishable from the steady state concentration within 5 days so k_2 must be greater than about 0.46 day^{-1} or $\tau_{½} = 1.5$ day. This means that in less than 1.5 days oysters are already half way to saturation. Alternatively, after having reached some value of naphthalene concentration, if allowed to depurate in water without naphthalene, half the sequestered naphthalene will be gone in less than 1.5 days. Again, we are assuming a one compartment model with overall uptake and elimination governed by first-order kinetics.

Therefore, stating k_2 is greater in oysters than in SPMDs for all PAHs is equivalent to saying

1. PAH concentrations in oysters more rapidly approach and retreat from steady state,
2. PAH concentrations in SPMDs are more readily retained when ambient concentrations decline than in oysters.

If an integrated view of the environmental exposure is desired, SPMDs will be effective or integrative over a greater period of time than oysters. This will be elaborated on in a later section.

UPTAKE AND CLEARANCE RATE CONSTANTS

A nonlinear regression was performed with the uptake data from the 250 ppt exposure to establish the rate constants k_1 and k_2. The equation regressed against the data was modified from the single compartment model to

$$C_t = \left(\frac{z}{k_2}\right)\left[1 - e^{-k_2 t}\right]$$

(Equation 4-7)

Note that z represents the product of the water concentration, W, and k_1. Fitting the equation in this way allowed determining the product of k_1 and W (as z) without first applying the (constant) exposure water concentration as either the nominal or measured value. The uptake constant was then determined by dividing by the water concentration deemed most appropriate. Oyster dry weight values were used (however lipid weighted k_1 values can be determined by the appropriate conversion factor as a good approximation to values obtained by regressing the data on that basis). Appendix C gives the regression results, covariance of the constants, and plots of the actual data and results predicted from the regression. The kinetic behavior of PAH uptake by oysters and SPMDs was classified into three groups; those having reached steady state in less than 5 days, or depending on whether k_2 was greater than or less than 0.01 day^{-1} as curvilinear or linear, respectively[2]. Using the measured water concentrations, k_1 was calculated and when possible oyster BCFs and SPMD Ks. SPMDs exhibited linear uptake for most PAHs during the exposure period so k_2 and K could not be calculated for the majority of PAHs. However, earlier work by Petty et al., can be used to estimate SPMD k2 values if one assumes no difference between the PAH partitioning in fresh water and sea water (Petty, Huckins et al. 1994).

[2] These assignments are based on arguments given in the Mathematical Appendix.

Oyster Constants

As suggested earlier, several lower molecular PAHs reached steady state in oysters in less than 5 days so only BCF values were calculated, Table 4-1. Higher molecular weight PAH uptake kinetics were very close to linear so uncertainties in k_2 became large and when convoluted with the larger uncertainties in PAH water concentrations resulted in lower confidence in the calculated BCFs. A better rule for the "Linear" classification for oyster data may be k_2 values less than 0.02 day^{-1}, due to large variance in the measured values resulting from the biological variation associated with organisms. The average uptake rate constant (k_1) was approximately 2.5 L/day/g (±37%). This data (Table 4-1) suggests that uptake is relatively constant and that k_2 determines BCF. An approximately linear relationship exists for the logarithm of the oyster PAH BCF versus the logarithm of the K_{ow} up to a log K_{ow} of about 6 (Figure 4-5). PAHs with K_{ow}s higher than 10^6 show a declining BCF. The large uncertainties for BCFs of PAHs beyond pyrene due to the low confidence of the k_2 and water concentrations may underestimate the BCFs of these higher molecular weight PAHs.

PAH Biological Half-lives. Although there are large uncertainties in many of the calculated k_2 values inferred from the uptake data of this study, a comparison can be made to the few values presented or derived from the literature, Table 4-2. In general, the agreement for bivalve half-lifes is quite good (even across species) with the exception of benz[a]anthracene which is much higher than the other cited values. The overall trend of an maximum in PAH half-life for the mid molecular weight PAHs with log K_{ow}s near 5.5 and a decline for those with log K_{ow}s beyond 6 appears in . Benzo[e]pyrene and benzo[a]pyrene half-lives estimated in this study are closer to those calculated by Bender et al., (Bender, Hargis et al. 1988) than those of Sericano et al., (Sericano, Wade et al. 1996) and do not show a difference between the isomers. Particularly encouraging is the agreement of the values for indeno[1,2,3-c,d]pyrene and benzo[g,h,i]perylene which are have low confidences. The possible influence of particulates on the derived half-lives was not discussed in either oyster study.

Table 4-1 Oyster PAH kinetics and rate constants calculated from the results of the 250 ppt exposure: k_1 has units of liters/day/g dry weight; k_2 is day^{-1}; the BCF is expressed on a mass basis (g water per g dry weight is adjusted by the density of seawater; "nd" indicates not determinable and relative covariance as percent is enclosed in parenthesis

Compound	Kinetics	k_1	k_2	BCF
naphthalene	Steady State	nd	nd	707 (18%)
benzo[b]thiophene	Steady State	nd	nd	439 (14%)
2-methylnaphthalene	Steady State	nd	nd	2350 (14%)
1-methylnaphthalene	Steady State	nd	nd	2160 (13%)
biphenyl	Steady State	nd	nd	3190 (13%)
1-ethylnaphthalene	Curvilinear	1.84 (30%)	0.314 (28%)	5980 (57%)
1,3-dimethylnaphthalene	Curvilinear	2.07 (26%)	0.281 (23%)	7520 (48%)
acenaphthylene	Steady State	nd	nd	1930 (15%)
acenaphthene	Steady State	nd	nd	3810 (14%)
4-methylbiphenyl	Curvilinear	1.76 (19%)	0.187 (19%)	9600 (38%)
2,3,5-trimethylnaphthalene	Curvilinear	2.47 (18%)	0.136 (20%)	18600 (38%)
fluorene	Curvilinear	1.85 (26%)	0.314 (23%)	6010 (49%)
1-methylfluorene	Curvilinear	2.52 (21%)	0.136 (19%)	18900 (39%)
dibenzothiophene	Curvilinear	2.58 (21%)	0.194 (19%)	13600 (40%)
phenanthrene	Curvilinear	2.67 (19%)	0.174 (16%)	15600 (36%)
anthracene	Curvilinear	2.52 (23%)	0.13 (17%)	19800 (41%)
2-methylphenanthrene	Curvilinear	3.21 (22%)	0.0553 (28%)	59200 (49%)
9-methylanthracene	Curvilinear	3.22 (16%)	0.0369 (27%)	89000 (43%)
3,6-dimethylphenanthrene	Curvilinear	4.55 (20%)	0.0191 (57%)	243000 (76%)
fluoranthene	Curvilinear	3.58 (23%)	0.0466 (35%)	78300 (58%)
pyrene	Curvilinear	3.7 (24%)	0.0365 (42%)	103000 (66%)
2-methylfluoranthene	Curvilinear	4.77 (27%)	0.0121 (101%)	402000 (128%)
benzo[b]naphtho[2,1-d]thiophene	Curvilinear	2.73 (55%)	0.0169 (173%)	165000 (228%)
benz[a]anthracene	Curvilinear	2.77 (54%)	0.0137 (201%)	206000 (256%)
chrysene	Curvilinear	1.95 (61%)	0.029 (116%)	68500 (177%)
benzo[b]fluoranthene	Curvilinear	3.3 (63%)	0.0287 (125%)	117000 (188%)
benzo[k]fluoranthene	Curvilinear	1.91 (76%)	0.0358 (139%)	54500 (214%)
benzo[e]pyrene	Curvilinear	2.33 (65%)	0.0317 (118%)	75000 (183%)
benzo[a]pyrene	Curvilinear	1.79 (84%)	0.0291 (192%)	62700 (276%)
perylene	Curvilinear	1.21 (76%)	0.03 (160%)	41100 (236%)
3-methylcholanthrene	Curvilinear	1.69 (127%)	0.103 (110%)	16700 (237%)
indeno[1,2,3-c,d]pyrene	Curvilinear	1.59 (102%)	0.0484 (173%)	33600 (275%)
dibenz[a,h]anthracene	Curvilinear	1.24 (104%)	0.0721 (126%)	17500 (230%)
benzo[g,h,i]perylene	Curvilinear	1.38 (88%)	0.0617 (114%)	22800 (203%)

Table 4-2 PAH Half-lives in oysters and mussels (days); *Crassostrea gigas* in this study, *Crassostrea virginica* values from Bender et al. (Bender, Hargis et al. 1988), Sericano et al. (Sericano, Wade et al. 1996) in oysters from chronically contaminated (CCon) and "uncontaminated" (UnCon) sites, and mussel (*Mytilus edulis*) values from Pruell et al. (Pruell, Lake et al. 1986).

Compound	Oysters This study *C. gigas*	Oysters Bender (1988)	Oysters Sericano (1996) UnCon	Oysters Sericano (1996) CCon	Mussels Pruell (1986)
naphthalene	< 1.8				
benzo[b]thiophene	< 1.8				
2-methylnaphthalene	< 1.8				
1-methylnaphthalene	< 1.8				
biphenyl	< 1.8				
1-ethylnaphthalene	2.2				
1,3-dimethylnaphthalene	2.5				
acenaphthylene	< 1.8				
acenaphthene	< 1.8				
4-methylbiphenyl	3.7				
2,3,5-trimethylnaphthalene	5.1				
fluorene	2.2				
1-methylfluorene	5.1				
dibenzothiophene	3.6				
phenanthrene	4	3.4			
anthracene	5.3				
2-methylphenanthrene	13	6.7			
9-methylanthracene	19				
3,6-dimethylphenanthrene	36				
fluoranthene	15	5.9	26	32	30
pyrene	19	6.7	10	12	--
2-methylfluoranthene	58				
benzo[b]naphtho[2,1-d]thiophene	41				
benz[a]anthracene	51	15	13	15	18
chrysene	24	15	12	16	14
benzo[b]fluoranthene	24	77			
benzo[k]fluoranthene	19				
benzo[e]pyrene	22	30	12	16	14
benzo[a]pyrene	24	22	9	10	15
perylene	23	9.2			
3-methylcholanthrene	6.7				
indeno[1,2,3-c,d]pyrene	14		10	11	16
dibenz[a,h]anthracene	9.6				
benzo[g,h,i]perylene	11	12			

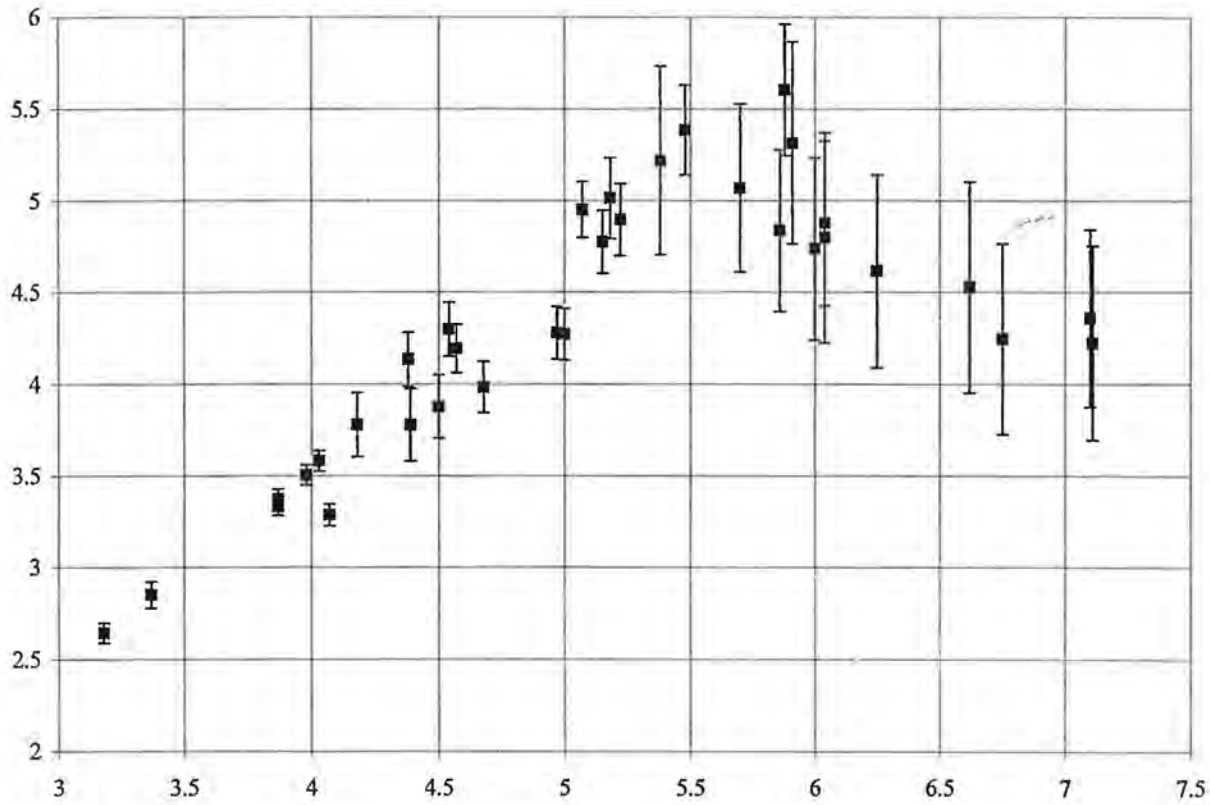

Figure 4-5 Log_{10} of the calculated BCF (y-axis) versus log_{10} of the PAH K_{ow} (x-axis)

SPMD Constants

The majority of the PAHs had k_2 values less than 0.01 day^{-1} and so behaved linearly over the course of the exposure (Table 4-3). Much lower variance appeared in all calculated k_1 values than for the oysters but this error rapidly increased as k_2 approached the 0.01 day^{-1} limit. An estimate of the SPMD - water partition constant can be made only for a few of the compounds which exhibited curvilinear behavior.

Table 4-3 SPMD PAH kinetics and rate constants calculated from the results of the 250 ppt exposure: k_1 has units of liters/day/half HUCK SPMD; k_2 is day^{-1}; the K is the estimated SPMD-water partition constant on a mass basis (mass of water per half HUCK SPMD); "nd" indicates not determinable and relative variance as percent is enclosed in parenthesis

Compound	Kinetics	k_1	k_2	K
naphthalene	Curvilinear	0.269 (13%)	0.177 (11%)	1550 (24%)
benzo[b]thiophene	Curvilinear	0.542 (17%)	0.237 (14%)	2340 (31%)
2-methylnaphthalene	Curvilinear	1.1 (15%)	0.0653 (21%)	17100 (37%)
1-methylnaphthalene	Curvilinear	1.05 (14%)	0.0676 (21%)	15800 (35%)
biphenyl	Curvilinear	1.06 (14%)	0.0505 (27%)	21400 (41%)
1-ethylnaphthalene	Curvilinear	1.2 (12%)	0.0213 (42%)	57400 (55%)
1,3-dimethylnaphthalene	Curvilinear	1.24 (13%)	0.0108 (75%)	117000 (87%)
acenaphthylene	Curvilinear	0.983 (18%)	0.0651 (25%)	15400 (42%)
acenaphthene	Curvilinear	1.11 (12%)	0.0377 (23%)	30000 (35%)
4-methylbiphenyl	Linear	1.18 (10%)	nd	nd
2,3,5-trimethylnaphthalene	Linear	1.27 (6%)	nd	nd
fluorene	Curvilinear	1.14 (13%)	0.0194 (46%)	60000 (59%)
1-methylfluorene	Linear	1.21 (10%)	nd	nd
dibenzothiophene	Linear	1.21 (12%)	nd	nd
phenanthrene	Linear	1.26 (12%)	nd	nd
anthracene	Linear	1.3 (14%)	nd	nd
2-methylphenanthrene	Linear	1.38 (12%)	nd	nd
9-methylanthracene	Linear	1.83 (12%)	nd	nd
3,6-dimethylphenanthrene	Linear	1.48 (12%)	nd	nd
fluoranthene	Linear	1.35 (13%)	nd	nd
pyrene	Linear	1.37 (15%)	nd	nd
2-methylfluoranthene	Linear	1.47 (19%)	nd	nd
benzo[b]naphtho[2,1-d]thiophene	Linear	1.31 (36%)	nd	nd
benz[a,h]anthracene	Linear	1.3 (37%)	nd	nd
chrysene	Linear	0.845 (40%)	nd	nd
benzo[b]fluoranthene	Linear	1 (40%)	nd	nd
benzo[k]fluoranthene	Linear	0.754 (46%)	nd	nd
benzo[e]pyrene	Linear	0.886 (43%)	nd	nd
benzo[a]pyrene	Linear	0.905 (48%)	nd	nd
perylene	Linear	0.934 (48%)	nd	nd
3-methylcholanthrene	Linear	3.7 (74%)	nd	nd
indeno[1,2,3-c,d]pyrene	Linear	1.02 (50%)	nd	nd
dibenz[a,h]anthracene	Linear	0.885 (58%)	nd	nd
benzo[g,h,i]perylene	Linear	0.885 (52%)	nd	nd

COMPARISON OF SPMDS AND OYSTERS

Basis of Comparison

The oyster and SPMD uptake rate constants (k_1s) provide a measure of sequestering "speed", the bioconcentration factors (BCFs) or partition constants (Ks) indicate the ultimate sequestering capacity, and time integrative capacity is determined by the clearance constants, k_2s.

Capacity: BCFs & Ks

As seen in Figure 4-5, oyster BCFs correlate well with PAH K_{ow} up to $\log_{10} K_{ow}$ 5.5. Regressing the oyster PAH (dry weight) BCFs against K_{ow}s up to a log K_{ow} of 5.5 gives a nearly linear relationship -

$$\text{Log BCF} = (1.14) \log K_{ow} - 1.09 \qquad R^2 = 0.94$$

(Equation 4-8)

and suggests the ultimate PAH capacity is proportional to the K_{ow} up to log K_{ow}s near 6. Note that this proportionality is not dependent on the basis of dry or lipid weighting - only the intercept is effected by this. Similarly, triolein-water partition coefficients (K_{tw}s)[3] have also been shown to be directly proportional to K_{ow}s (Chiou 1985);

$$\log K_{tw} = 1.00 \log K_{ow} + 0.105$$

(Equation 4-9)

Huckins et al. has shown the similarity between SPMD K_{tw}s and Chiou's K_{tw}s . The conclusion is that oyster and SPMD PAH capacities are proportional to PAH K_{ow}s up to K_{ow}s of approximately 10^6 regardless of how the concentrations are expressed and sequestered concentrations are related to ambient water concentrations. Oysters, and marine bivalves in general, have roughly 1 to 4 % lipid or 100 to 250 milligrams per individual compared to SPMDs

[3] The liquid-liquid partition coefficient, no membrane involved.

which contain 0.5 to 1 g triolein (≈ 20% triolein by mass). Obviously more samplers will provide more capacity and more signal.

Uptake Rates[4]

The average k_1 for the oysters is approximately 2.5 L/day/g dry weight or approximately per oyster and are comparable to the ½HUCK rate of about 1L/day. Combining oysters and deploying more SPMDs can be used to increase the volume of water sampled and analytical signal, however, cleanup of large amounts of oyster tissue quickly becomes problematic due to the inherent difficulties of removing complex interferences coextracted from biological matrices. Uptake rates in oysters increase with PAH molecular weight (and K_{ow}) until methylfluoranthene (#22) then apparently rapidly decline, Figure 4-6. While oyster k_1 values show no bias between the 3 ring PAH isomers, phenanthrene (#15) and anthracene (#16), some preferential sampling biases are implied for isomers with 4 or 5 rings; i.e., pyrene (#21), benzo[a]anthracene (#24) and chrysene (#25); benzo[b]fluoranthene (#26) and benzo[k]fluoranthene (#27). However, variance is high and prevents a high degree of confidence in these observations. However the apparent difference between chrysene (#25) and the more branched benzeniod isomers seems justifiable on the basis of the extreme change in molecular size.

[4] Ideally,uptake rate constant comparison for oysters and SPMDs should be based on units of L day^{-1} g^{-1}. In both cases matrix mass, g, should represent the whole sample weight (oyster wet weight and SPMD membrane plus lipid). Unfortunately, the variability of the measurements of oyster wet weight precludes this standard comparison. Thus comparions were made on the basis of one oyster (about 1 g dry weight) to one half HUCK SPMD as used in the exposures.

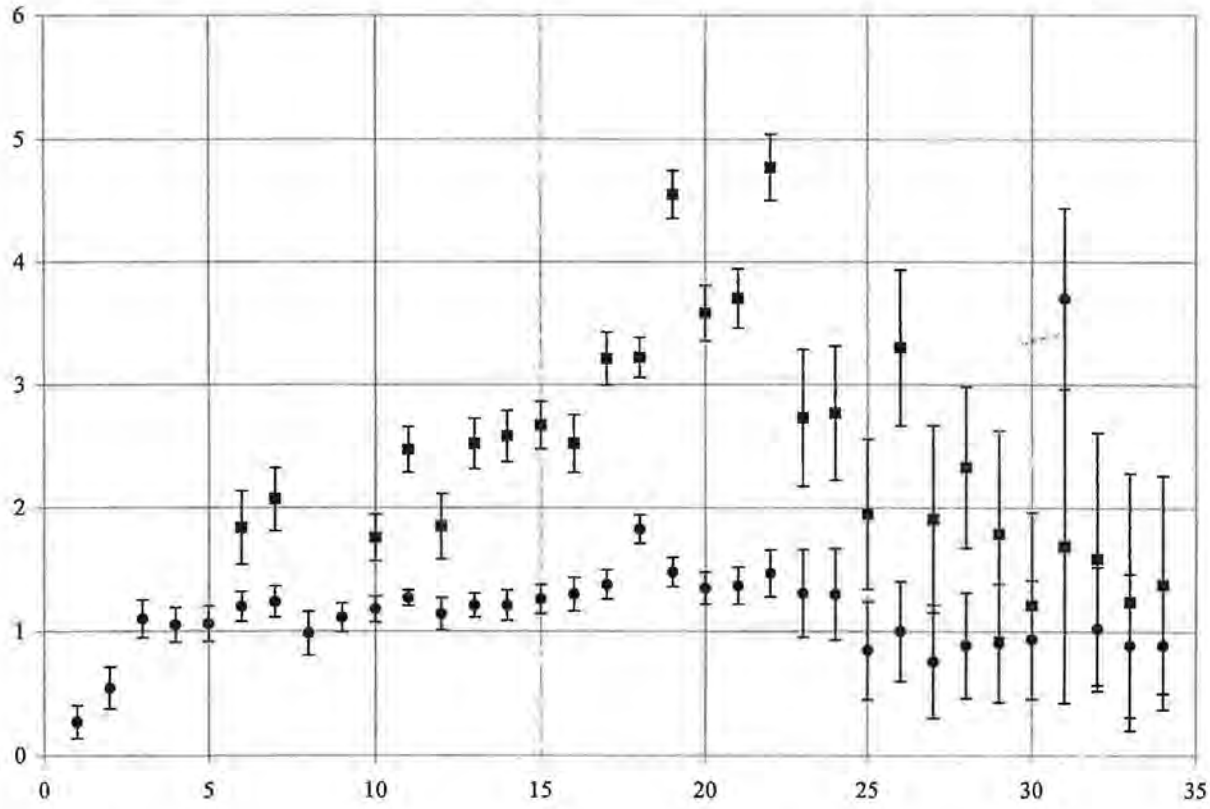

Figure 4-6 Uptake rates for PAHs by oysters and SPMDs in the 250 ppt Exposure: Oyster k_1 values (squares) are in L/day/g dry weight; SPMD k_1s (circles) are in L/day/½ HUCK SPMD and are uncorrected by the PRC loss. See text and Table 1-1 for compound index numbers (x-axis). Compound #31, 3-methylcholanthrene, values are highly suspect due to analytical problems.

SPMD values are uncorrected for permeation reference compound recovery (Appendix D) which is expected to increase k_1 values. The most profound change is exhibited in the change from the 2 ring PAHs naphthalene and benzo[b]thiophene to higher K_{ow} compounds. The only statistically significant evidence for an isomeric sampling bias is for 2-methylphenanthrene (#17) and 9-methylanthracene (#18). The increase for 9-methylanthracene is interesting and suspicious since it is also higher than sampling for the dimethylphenanthrene (#19) and therefore can not be explained simply on the basis of an increase in K_{ow}. (Note that (#17) and (#19) do not differ significantly). Molecular size is a possible explanation for the difference between pyrene (#21) and PAHs with molecular weights greater than or equal to chrysene (#25).

Correspondence between SPMDs and Oysters: "Scaling Factors"

The relationships between the logs of the BCF and K_{tw}, (Equation 4-8) and (Equation 4-9), and the definitions of BCF and K in terms of rate constants suggest a logarithmic connection or scaling between the uptake rate constants for oysters and SPMDs -

$$\log k_1^{oyster} = (A)\log k_1^{SPMD} + F(k_2^{oyster}, k_2^{SPMD}) + D$$

(Equation 4-10)

where A and D are constants, and $F(k_2^{oyster}, k_2^{SPMD})$ implies some function of the oyster and SPMD clearance constants. If $F(k_2^{oyster}, k_2^{SPMD})$ is relatively consistent among compounds, there should be a linear relationship between the log of the oyster and SPMD uptake constants. Figure 4-5 indicates the linear relationship between BCF and K_{ow} maintains to a log K_{ow} of approximately 5.5. The regression of the log of the oyster uptake constants against and the log of ½HUCK SPMD k_1 values against the SPMD k_1 values for PAHs with K_{ow}s $< 10^{5.5}$ appears linear, Figure 4-7. The slope is 3.63 and the intercept 0.0341 with an R^2 of 0.85; i.e.,

$$\log k_1^{oyster} = (3.63)\log k_1^{SPMD} + .0341$$

The most pronounced exception to this trend is 9-methylanthracene; uptake of the methylanthracene by oysters is comparable to that of the methylphenanthrene but uptake by SPMDs is roughly 30% higher for the methylanthracene. This suggests limits to the correspondence however, passive uptake seems to apply to oysters. In the cases where this is true, SPMDs offer a meaningful surrogate or predictor with respect to exposure kinetics and bioconcentration. The relationship between the logs of the uptake constants suggests there may be a correspondence between k_2 values (Equation 4-10) which should also be explored.

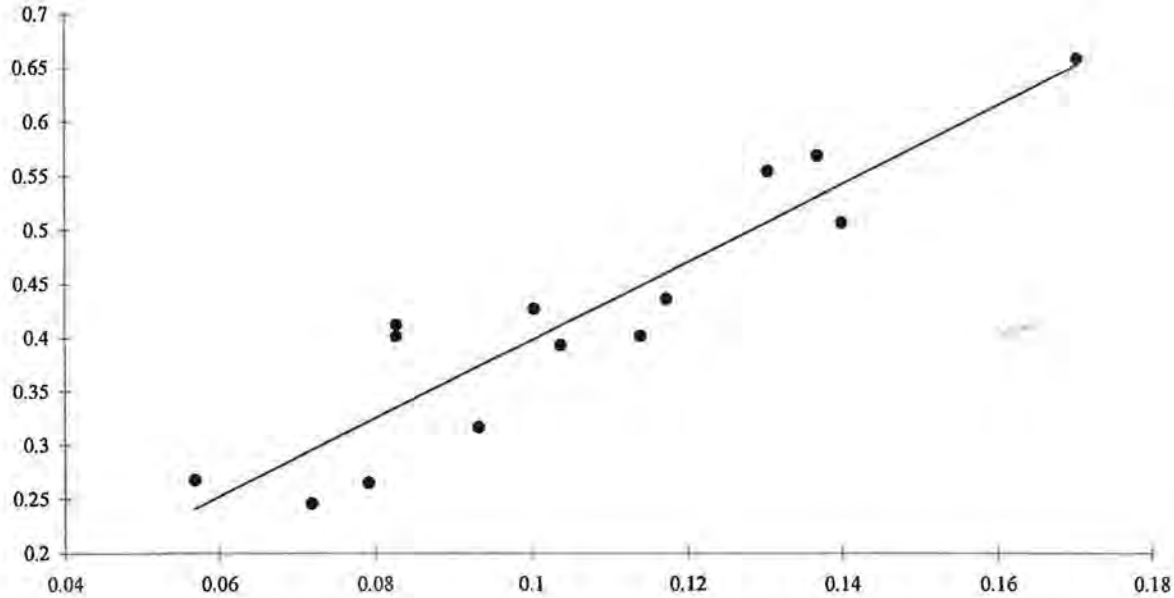

Figure 4-7 Scatter plot of the $\log_{10}$ of the calculated oyster (y-axis) and ½HUCK SPMD (x-axis) PAH uptake rate constants for 14 PAHs with log K_{ow}s < 5.5 and the resulting linear regression; $R^2 = 0.852$. (9-methylanthracene was not included)

Chapter 5
CONCLUSIONS

SUMMARY

The present study represents the first laboratory comparison of SPMDs and oysters exposed to PAHs and has demonstrated the superior reproducibility of SPMDs and inherent differences in the capacity of oysters and SPMDs as environmental monitors due to kinetic limitations.

These are the first bivalve exposures designed to quantify dissolved-phase uptake of hydrocarbons. By prefiltering the sea water to below the filtration limit of the oysters, the particulate contribution to the residue uptake was eliminated unlike earlier studies. Most previous studies used open exposure systems and consequently ambient concentrations were poorly defined and/or characterized and particulate removal did not extend below the filtration limit of the bivalves. The oyster PAH profiles were similar to those observed in field deployments in spite of the fact that particulate contributions are expected to play a significant role (Prest, unpublished data). A connection between SPMDs and oysters in field situations, essentially a connection between the dissolved phase and oyster tissue concentrations, seems possible for light to mid-molecular weight PAHs which are not expected to be strongly bound to particulates if concentrations remain constant over the course of the exposures. At this point, based on the (Chapter 4) discussion of oyster BCF and K_{tw}, and the correlation between uptake rate constants, SPMDs appear to be large (with respect to capacity) and slowly responding (with respect to kinetics) oyster surrogates. Field work with water sampling will be required to strengthen the analogy.

Analytical and Practical Issues

The analytical chemist's criteria for sample detection and quantitation is based on the signal-to-noise ratio. Ultimately the environmental monitor must be reproducible and sensitive. SPMD reproducibility is on average 5 times better than oysters. SPMD relative standard deviation was as low as 1% for the low molecular weight PAHs and reached 20% for the late eluting, high

molecular weight PAHs which are typically difficult to analyze. Replication, as measured by RSDs, of low molecular weight PAHs in oysters was on the order of 9 to 11%, and reached 25% to 50% for "high end" PAHs. Also RSDs were inconsistent among exposure sets. The implications are that site to site differences in sources will be more difficult to distinguish via oyster monitoring than by SPMD analysis. Combining oysters to form a composite sample can improve the statistical situation but will never reduce the variance beyond the limitation imposed by the individual, living organisms. However, the high variation of individual oysters inherently prevents a strong correlation between oyster concentrations and ambient concentrations, even in the ideal scenario where only the dissolved phase is involved.

Time integrative capacity - practical considerations and implications of the rate constants

Because the clearance constant, k_2, is larger in oysters than in SPMDs for all PAHs, the response to changes in ambient concentration must be faster in oysters than in SPMDs and weakens their ability to provide a time weighted average record of variable ambient concentrations and of transient contamination events.

In characterizing these kinetic constants we have gained some insight into how oyster and SPMD concentrations relate to their common experience in the environment. Because k_2 is smaller in oysters than in SPMDs for all PAHs, oyster tissue concentrations have a shorter "memory" for encounters of PAH contamination than SPMDs. A "real world" example will help to clarify the situation.

Imagine an event that introduces a contaminant into the environment (such as spill) but the component rapidly dissipates from the water in the vicinity of the monitors (e.g., dilution or flow, volatilization or other removal). Our interest is first to detect the compound's presence and second, determine the average concentration of the contaminant; make an assessment of the exposure. Knowing the constants determined earlier (Chapter 4) allows a simulation of the response of oysters and SPMDs to such a transient input of chemical[1]. Since k_1 and k_2 for 1-

[1] The simulation requires simultaneously solving several differential equations using numerical methods. This simulation was performed using Gepasi© release 2.08 by Pedro Mendes with oyster uptake and clearance constants as 1.84L/day/g and

ethylnaphthalene were determined for both oysters and SPMDs, we will model this analyte concentration. Figure 5-1 shows the simulated results of a short exposure (of less than one day) to 1-ethylnaphthalene. The maximum concentrations are reached in about a day and then begin to decline since the source has disappeared. Because k_1 is large in the oysters a high level is rapidly reached, however, since k_2 is also large the half-life is short ($t_{½} = \ln 2 / k_2 = 2.2$ days). From a high of approximately 40 ng/g at the end of day one, by 2.2 days later, the remaining ethylnaphthalene tissue levels are down to about 20 ng/g and a negligible amount remains by day 15. Since each oyster is about one gram dry weight, this is the response expected by each individual oyster.

The SPMD responds similarly reaching a maximum concentration of 31 ng/½HUCK in the first day, the important difference is the slower loss from the SPMD due to the lower k_2. The half-life of ethylnaphthalene is about 32.5 days so in 34 days there will still be roughly 15 ng/½HUCK.

0.314/day; SPMD constants 1.2 L/day/half HUCK and 0.0213/day; [A]initial was 100 ng with a dissipation rate of 0.693/day. Using the 250 ppt study values for the constants and the water concentration, Gepasi reproduced the results of the non-linear regression within the uncertainties.

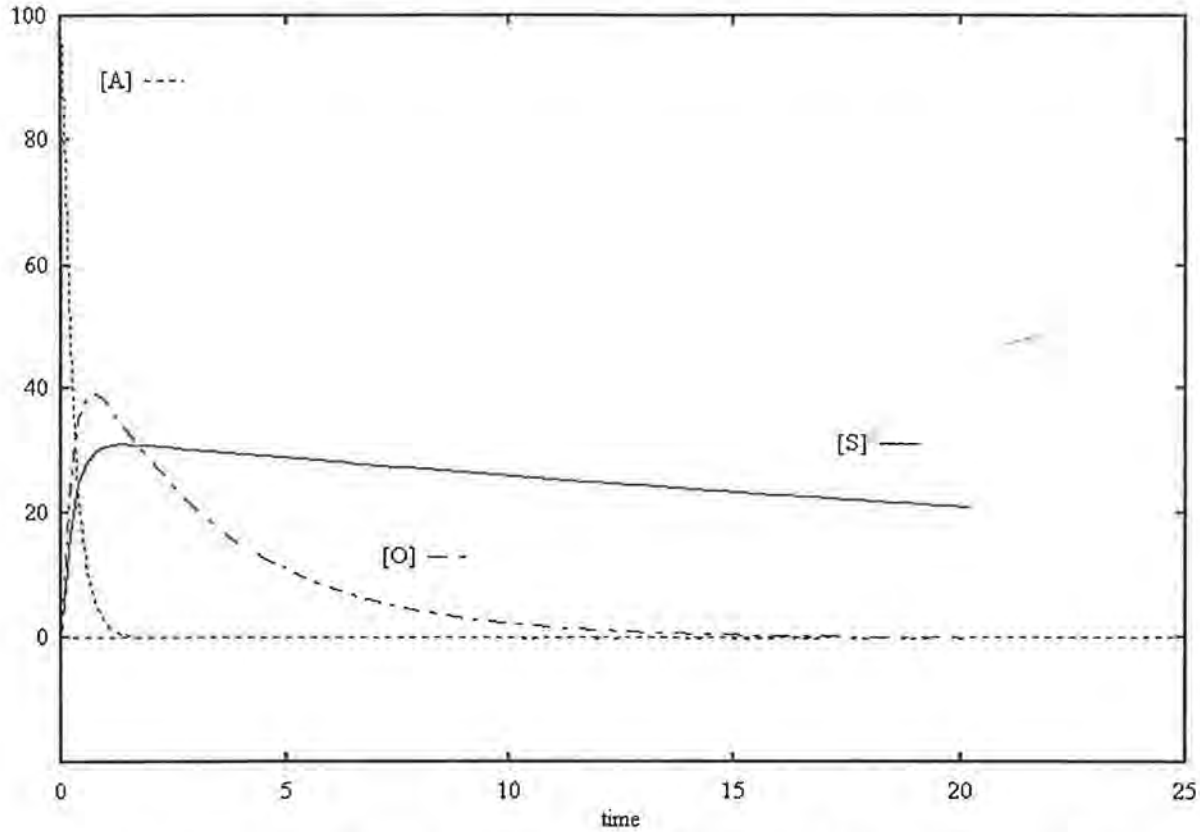

Figure 5-1 "Spill" exposure of 1-ethylnaphthalene [A] and the accumulation in oysters [O] and SPMDs [S] versus time in days as ng per gram dry wt in oysters and ng per half HUCK SPMD (y-axis). The initial exposure of 100 ng/L has a half-life or duration of less than a half day.

In any practical comparison, analytical detection limits must be considered. Assuming 20 ng / (dry) g oyster and 20 ng/½HUCK are measurable levels, then after 3 days none can be detected in the oysters as opposed to 21 or 22 days in the SPMDs. To improve detection limits, more environmental monitors can be used (assuming background residues are low, which is easier to achieve for SPMDs than bivalves). Figure 5-2 show results of a simulation of a transient introduction of ethylnaphthalene near the solubility limit of 10 mg/L monitored by 10 oysters and a one (HUCK) SPMD[2]. If detection limits are 20 ng / g dry weight oyster and 20 ng/½HUCK, then 200 ng are detectable in 10 oysters and similarly 40 ng will be detectable for the SPMD. Ethylnaphthalene will be below the detection limits in the oysters after 11 days while the residue in the SPMD will require approximately 140 days to decrease below detection limits. **Adding**

[2] We assume that the bivalves continue to sequester at the same rate as calculated from lower exposures even though data shows (ref Chapter 1) beyond about 500 µg/L uptake decreases dramatically. Similarly, we assume SPMD uptake is unchanged.

more monitors as a strategy to improve contaminant measurement effects only the total uptake, <u>not</u> k_2 ; thus only enhancing detection limits can extend the range beyond the inherent limitation imposed by the clearance constant. Realistically, only so much material can be cleaned up. This study suggests that oyster PAH k_2s are at least 15 times greater than those for SPMDs so PAH half-lifes and times for detection must always be at least 15 times less in oysters.

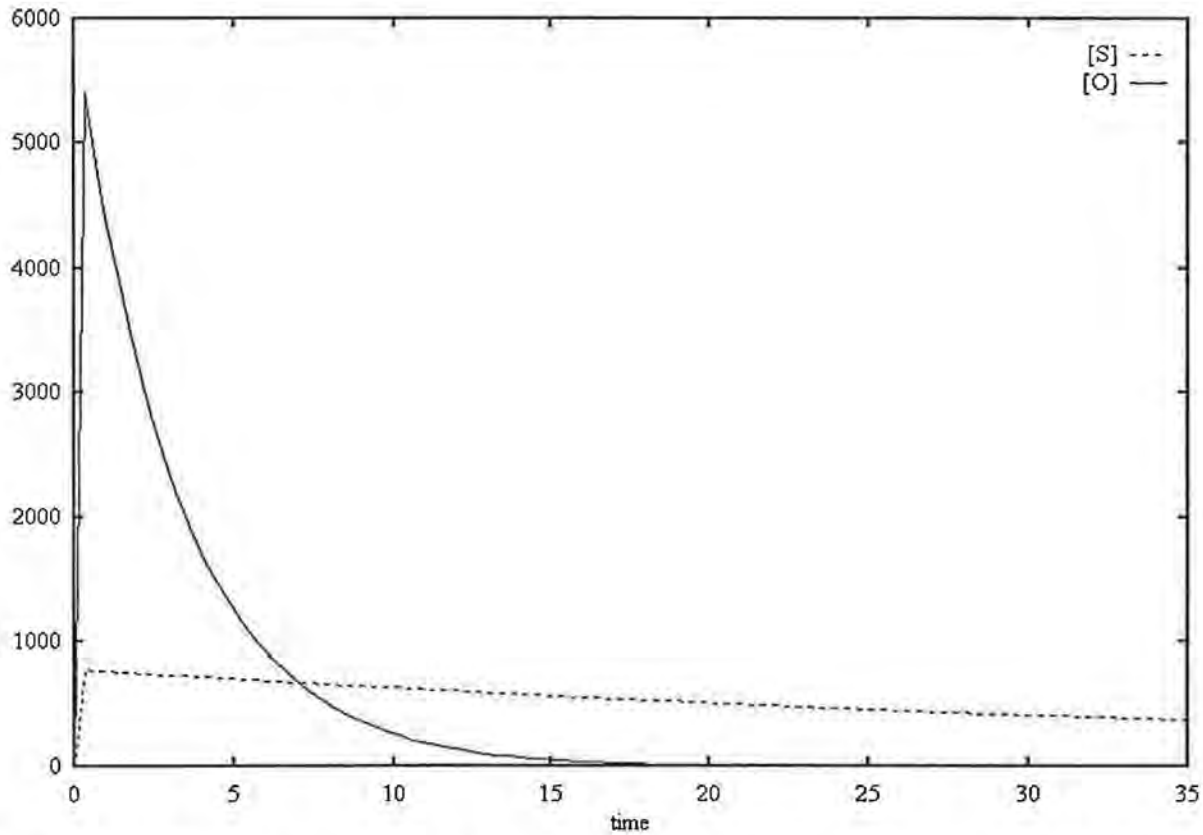

Figure 5-2 "Spill" exposure of 1-ethylnaphthalene (not shown) and the accumulation in oysters [O] and SPMDs [S] versus time in days as ng sequestered by 10 oysters and ng per one HUCK SPMD (y-axis). The initial exposure of 10,000 ng/L (near the solubility limit) has a half-life or duration of less than 1.7 hours.

FUTURE RESEARCH AND APPROACHES

Field comparisons

The influence of particulates on the oyster profiles should be accessed. The presence of high molecular weight PAHs which are most likely to be particulate bound and have a higher BCF than the low molecular weight compounds are expected to change the shape of the profile if they are assimilated into the tissue. Direct measurement of clearance rates for individual PAHs in both SPMDs and oysters in a side-by-side study will provide a higher degree of confidence in the k_2 values which are critical parameters for comparison of their abilities as environmental monitors.

Estimation of Water Concentrations from SPMDs

On the basis of the high reproducibility of data and the proportional response of SPMD concentrations to ambient PAH concentrations (Petty, Huckins et al. 1995; Petty, Huckins et al. 1995), the promise of accurately calculating water concentrations from passively sequestered SPMD concentrations is great. The most significant impediment involves correction for the boundary layer or biofouling effects. One promising approach utilizes a permeation reference compounds, PRCs (e.g., d10-phenanthrene in this study) to correct for the additional impedance to residue uptake (ref. Appendix D). The SPMD results presented herein were not corrected with PRC values. Therefore, it is likely that the SPMD k_1 values are underestimated and k_2 values are overestimated; a slight bias that favors the oysters in terms of PAH uptake and half-life comparisons. In any case, the essential results and arguments should not be affected. To globally acquire a sense of the problem and approaches to solutions consider the following simplified description.[3]

A diagram of the situation in Figure 5-3 shows the media involved, concentrations[4], gradients and related resistances. Essentially, the concentration of the analyte A in the water, $[A]_W$, is not

[3] The following description is vastly simplified for convenience but true in essence. The purpose is to physically illustrate the problem and a possible solution without resorting to differential equations.

[4] Actually concentration is not the appropriate term or "metric" for understanding the situation; chemical potential, activity or G.N. Lewis' idea of fugacity more accurately describe physical chemical situation. It is realized that, on a concentration basis, the triolein ($[A]_s$) rapidly exceeds the concentration in the surrounding water, $[A]_w$ so the "downhill" concentration gradient is

experienced at the surface of the SPMD polymer membrane due to the finite resistance of the boundary or biolayer, R_b. Based on the analogy with electric circuits, we can write the mass of analyte, M, transported per time per unit area as

$$dM/dt \propto \text{Concentration Gradient / Total Resistance}$$

(Equation 5-1)

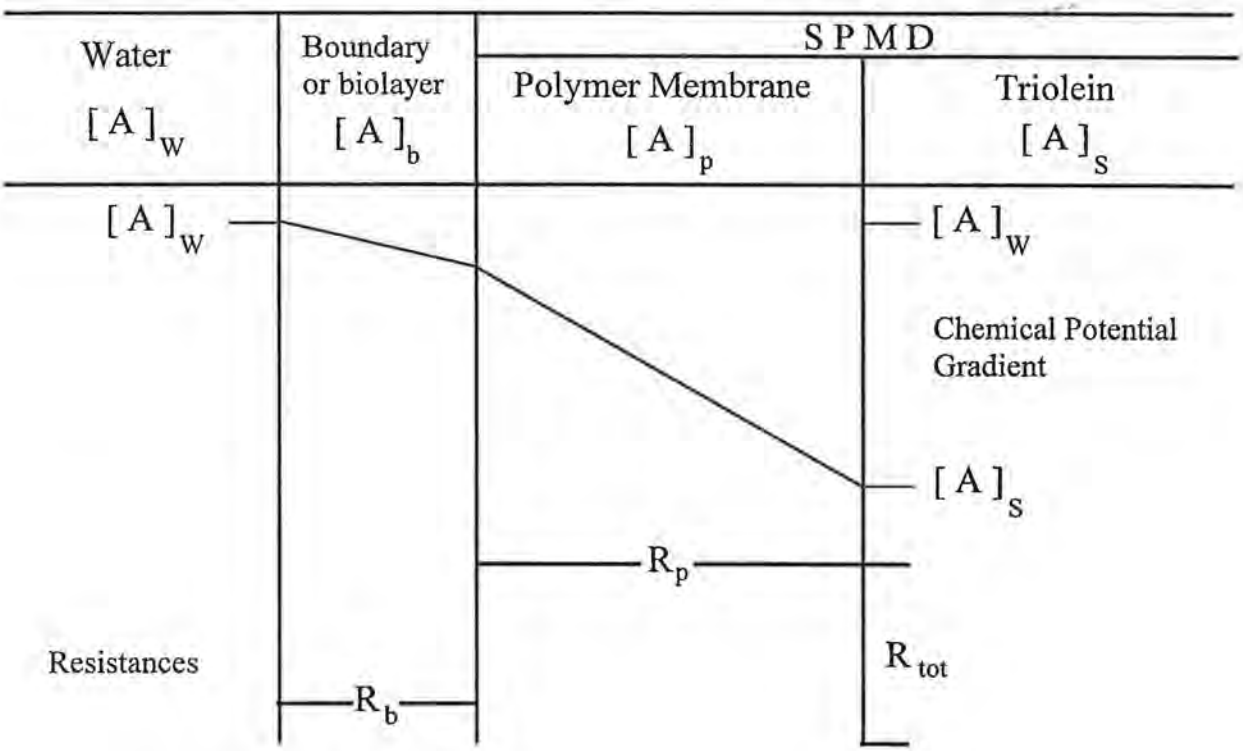

Figure 5-3 Diagram of the compartments important to SPMD kinetics. [A] represents the (activity) concentration of an analyte in water, the boundary or biolayer, polymer membrane or lipid compartment. The resistances to mass transport are indicated by R.

Because we can choose any appropriate unit for the resistance, we can write this as an equality and let the resistances be their averages over time so that the total mass transported or accumulated in the triolein in unit time per unit area, M, is

only true picture for a very short period of exposure. However, the chemical potential gradient favors the triolein until equilibrium when by definition the analyte potential in the water and SPMD are equivalent. So the reader may substitute "activity" for "concentration" if prefered.

$$M' = \frac{[A]_W}{R_b + R_p'}$$

(Equation 5-4)

Notice that the boundary or biolayer experienced in the exposure is the same[7] and only the polymer resistance has changed and this by design. We could have doubled the polymer thickness or whatever but there is some relationship between R_p and R_p';

$$R_p' = F\, R_p$$

(Equation 5-5)

For example, if the polymeric membrane is twice as thick, we can (approximately) arrange F = 2 and $R_p' = 2\, R_p$. The important point is that R_p' is different from R_p and well known. Then by taking the differences of the inverses of the sequestered amounts gives -

$$\frac{1}{M'} - \frac{1}{M} = \frac{R_b + R_p'}{[A]_W} - \frac{R_b + R_p}{[A]_W} = \frac{R_p' - R_p}{[A]_W}$$

(Equation 5-6)

If $R_p' = 2\, R_p$, we obtain

$$\frac{1}{M'} - \frac{1}{M} = \frac{R_p}{[A]_W}$$

(Equation 5-7)

or for the water concentration of A in terms of the known or measured quantities,

[7] Unless the biolayer is indirectly effected by the membrane design change; e.g., oleic acid flux to the surface changes the fouling rate.

$$[A]_W = \frac{R_p}{\left(\frac{1}{M'} - \frac{1}{M}\right)}$$

(Equation 5-8)

This approach takes advantage of the high analytical reproducibility of SPMDs. For example, if the SPMD analytical variation for a particular PAH is 5%, then the water concentration can be calculated to within a total uncertainty of 20%. It should be stated that only relative R_ps (k_1s) need be measured so the experimental complexity and uncertainty is greatly reduced. It may be possible to make permeation measurements of the polymer only.

The same considerations can be applied to the permeation reference compound and it can be shown that the PRC can be used to measure the average temperature experienced over the course of the deployment (if the PRC shows a significant variation of k_2 with temperature) and if necessary the resistance of the biolayer can be calculated. This approach provides key information for calculating water concentrations to be provided by measured SPMD concentrations. An excellent foundation is already in place for this approach which appears promising for calculating time integrated water concentrations.

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