SEDIMENT CHARACTERISTICS AND BIOAVAILABILITY OF SORBED NEUTRAL ORGANIC COMPOUNDS

THESIS

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Several sediment characteristics were analyzed to determine their suitability for use as potential normalization factors for the bioavailability of neutral organic compounds sorbed to sediments. Percent organic carbon, cation exchange capacity and particle surface area were measured sediment characteristics that varied sufficiently to encompass the range in observed sediment toxicity. Laboratory sediment toxicity test data using fluoranthene suggest that there is no biologically significant correlation between sediment toxicity and sediment characteristics (organic carbon, cation exchange capacity, particle size distribution, particle surface area). Fluoranthene amended sediments with similar organic carbon contents do not yield similar toxicities due to sorbed fluoranthene and thus do not support the organic carbon normalization approach for evaluating sediment quality or for sediment criteria development.

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CHAPTER I

INTRODUCTION

Recently, concern has been expressed about the ability of the U.S. Environmental Protection Agency's (EPA) Water Quality Criteria (WQC) to sufficiently ensure protection of aquatic life within the provisions of the Clean Water Act (Battelle, 1985). Degradation of aquatic systems has been noted in some areas where WQC have not been exceeded in recent history (JRB Associates, 1984; Lyman, 1987). In response to these concerns, EPA may develop numerical criteria for chemicals in sediments [(Sediment Quality Criteria (SQC)] and apply them to geographic locations containing sediments with significant amounts of potentially toxic materials.

The rationale for this approach is that concentrations of potentially toxic materials in sediments that are greater than proposed SQC would suggest that these sediments are potentially toxic or hazardous. Clearly, this could have a significant impact on industry. If SQC are underprotective, then many benthic communities could be lost. However, if SQC are too protective, then this could inhibit chemical development and possibly deprive society of potentially beneficial chemicals. First, bottom or consolidated

sediments serve as sinks for many potentially toxic materials (Lyman, 1987; Pavlou, 1987), and long-term effluent discharges could lead to toxic contaminant concentrations in the sediments of receiving freshwater, marine or estuarine systems. In addition, relatively large numbers of organisms live in and around bottom sediments. Therefore, sediment contaminant concentrations could be as important as water concentrations in establishing National Pollution Discharge Elimination System (NPDES) permit limits and accomplishing the goals of the Clean Water Act. Several approaches have been proposed for development of SQC.

Organic Carbon Normalization Approach

Organic carbon (OC) content of sediments has been proposed as a normalizing factor for bioavailability of neutral organic compounds sorbed to sediments (Shea, 1988). If a compound is bioavailable, then it should elicit some response from an organism. The organism's response is a function of exposure concentration and duration of exposure. The organic carbon normalization approach implies that the bioavailable fraction of a toxic material in a sediment is governed or moderated by the total organic carbon content of that sediment. According to the theory, as the OC content of the sediment increases, the toxicity of the contaminated sediment concomitantly decreases. Use of this normalizing

factor for SQC involves using the concentration of the toxic material in sediment as the numerator and using the OC content (%) as the denominator. The resultant number is hypothesized to be a closer approximation of the bioavailable concentration of the toxic material:

$$\frac{\text{TOXICANT CONCENTRATION IN SEDIMENT}}{\text{SEDIMENT OC(\%)}} = \text{ESTIMATED BIOAVAILABLE CONCENTRATION IN SEDIMENT}$$

There are at least two concerns with the organic carbon normalization approach for development of SQC. First, there is no established relationship between sediment toxicity and bulk chemistry or quantity of toxic materials in sediments (Battelle, 1985). The mere presence of potentially toxic materials in sediments does not guarantee that these sediments are toxic to aquatic life. Secondly, using OC as a normalizing factor for SQC may or may not be appropriate. An implicit assumption is that OC content in sediments varies at least two orders of magnitude because observed toxicity ranges from zero to 100 percent. Adams (1987) has shown that OC content of sediments collected across the U.S. has a mean of 2.0% and that 96% of the values were within two orders of magnitude (0.1 to 10%). Mathematically, if the OC content of sediment is less than 1%, then the bioavailable fraction of toxic materials would theoretically be greater than the

measured sediment concentration. It appears that organic carbon might not vary sufficiently in aquatic sediments for use as a normalizing factor as intended (Rodgers et al. 1987). A key to this dilemma is that sediments with similar OC contents and concentrations of toxic materials may vary widely in observed toxicity (from 0 - 100% mortality). This is addressed below.

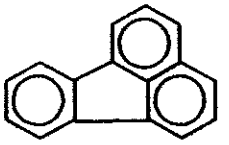
Sediment Characteristics as Potential Normalization Factors

Organic carbon content of sediments is the most recognized parameter suggested to control bioavailability of neutral organic compounds (Adams, 1987). However, other sediment characteristics have not received much attention as potential normalization factors. Kimerle (1987) stressed the importance of determining the effects of clay particles and other important factors on the availability of chemicals in sediments. Lyman (1987) lists particle size as a possible covariate with organic carbon in determining chemical partitioning in sediments. Malueg et al. (1987) raised the question whether or not physical characteristics of sediments such as grain size are important in sediment contaminant concentrations. For example, in contaminated New York Bight sediments, contaminant concentrations were strongly related to grain size and organic carbon content. Cation exchange capacity and organic carbon have also been

suggested to affect bioavailability of polar or ionizable organic compounds (Fava et al. 1987). Organic chemicals of petroleum origin that are relatively water insoluble should be good models to test hypotheses regarding regulation of toxicant bioavailability by sediment characteristics. Fluoranthene is an example of an organic chemical with a propensity to sorb to sediments that may be toxic at a fraction of its aqueous solubility.

Physical Properties of Fluoranthene

Fluoranthene belongs to a group of compounds known as polycyclic aromatic hydrocarbons (PAH's) which are fused compounds built on benzene rings. PAH's are only slightly soluble in water due to their high molecular weight and nonpolar hydrophobic nature (Figure 1). Solubility tends to increase as the number of aromatic rings or molecular weight decreases. PAH's have anywhere from two (low molecular weight) to five (high molecular weight) aromatic rings with fluoranthene having three rings. Thus fluoranthene is considered to be a lower molecular weight aromatic compound. Since fluoranthene is relatively water insoluble (150 ug/l: observed, in pond water) and is hydrophobic in nature, it sorbs readily to particulate matter upon entering an aqueous environment. These particulate materials then settle to bottom sediments where fluoranthene can accumulate to

Characteristics*	Fluoranthene
Structure	
Mol. Weight	202.3
Melting Point	111°C
Solubility in Deionized Water	206 - 265 ug/L
Observed Solubility (Pond Water)	150 ug/L
Observed Kp	1×10^3

*U.S. Environmental Protection Agency, 1979

FIGURE 1. Fluoranthene Characteristics.

concentrations orders of magnitude higher than overlying water due to its favorable partitioning to sediments. Low molecular weight PAH's such as fluoranthene are removed from the overlying water by volatilization, microbial oxidation and sedimentation (Moore and Ramamoorthy, 1984). However, fluoranthene deposited in sediments is less subject to biological or chemical breakdown, especially if the sediment is anoxic - thus fluoranthene is reported to be persistent in bottom sediments (Neff, 1979).

Sources of Fluoranthene

Fluoranthene originates from both natural and anthropogenic sources. Fluoranthene can be produced in small quantities in nature by plants, algae, bacteria, and other microorganisms, and is found in remote regions in water and sediment thus accounting for natural, low background concentrations (Moore and Ramamoorthy, 1984). PAH's can also be found in low concentrations in smoked foods, cigarette smoke, vegetable oils and margarine.

Fluoranthene and PAH's in general are not evenly distributed in the environment. Elevated PAH levels are usually associated with heavy industrial activity and high population densities. Fluoranthene is discharged into the air from engine exhaust emissions, coal combustion and forest fires. Sources of fluoranthene in surface waters

include municipal and industrial effluents (Harrison et al., 1975), atmospheric fallout, fly-ash precipitation, road run-off from bituminous road surfaces and tire wear, and oil spills.

Effects of Fluoranthene on Aquatic Organisms

Acute Toxicity

Very little research has been done concerning the acute toxicity of fluoranthene to freshwater organisms. However, acute toxicity of PAH's in general tends to increase as molecular weight increases. Most of the data generated to date have shown that crustaceans are the most sensitive species, followed by polychaete worms and fish (Neff, 1979). In most cases, the concentration of PAH needed to elicit an acute response in aquatic organisms is several orders of magnitude higher than the PAH concentration found in heavily polluted water bodies (Neff, 1979).

Chronic Toxicity

Very little, if any, data exist concerning the chronic effects of fluoranthene on freshwater organisms. However, chronic exposures of other PAH's in water and sediment may elicit sublethal responses in sensitive aquatic organisms. Chronic effects of other PAH's to aquatic invertebrates include reduced growth and molting rate, decreased

fecundity, and behavioral disorders such as locomotor impairment and abnormal burrow construction. Effects on fish include decreases in growth and fecundity and behavioral abnormalities (Moore and Ramamoorthy, 1984).

Fluoranthene Partitioning

Since EPA will apparently focus primarily on neutral organic compounds when initially developing sediment quality criteria, a neutral organic compound (fluoranthene) was used to amend the sediments in this study. Usually chemical partitioning is dependent upon its aqueous solubility (Reinert and Rodgers, 1987). Neutral organics with a high water solubility (>10 mg/L) tend to remain in the water column and do not sorb appreciably to sediments. But neutral organics with a relatively low water solubility (<5-10 mg/L) sorb more to sediments and will be found in much lower amounts in the water column. In addition to aqueous solubility, the partition coefficient (K_p) plays an important role in a neutral organic compound's ability to sorb to sediments and therefore be removed from overlying water (Staples et al., 1985). The sorption partition coefficient of a chemical is expressed as follows:

$$K_p = \frac{\text{chemical concentration in sediment}}{\text{chemical concentration in water}}$$

The partition coefficient can also be expressed as K_{oc}, which is normalized for organic carbon content and is expressed as follows:

$$K_{oc} = \frac{K_p}{\% \text{ OC}}$$

Generally speaking, neutral organics with a high K_p ($>10^7$) are not found in high concentrations in the overlying water, but mostly sorbed to sediments. Neutral organics with low K_p's ($<10^4$) have a greater affinity for the overlying water and sorb little to sediments. Therefore, compounds with a high aqueous solubility and low K_p (e.g. phenols) will most likely be found in the water column and their toxicity could be accurately detected by aqueous phase testing methods. On the other hand, compounds with a low water solubility and high K_p (e.g. PCB's and fluoranthene) will be sorbed mostly to the sediment and their toxicity may be detected with sediment toxicity test methods.

Acute vs. Chronic Effects of Chemicals

Whether or not neutral organics exhibit acute or chronic toxicity depends upon the compound's K_p, water solubility, structure, and mode of action. Neutral organics with a high K_p and low water solubility will tend to exhibit chronic toxicity while neutral organics with a low K_p and

high water solubility will often demonstrate acute toxicity.

However, there are chemicals of environmental consequence and concern that have low aqueous solubilities and exhibit acute toxicity (e.g. fluoranthene, anthracene). These chemicals' acute toxicity is usually a small fraction of their aqueous solubility. Consequently, these chemicals could exhibit acute toxicity even if very small amounts are released from the sediments into the interstitial and overlying water.

Research Objectives

The objectives of this research are:

1) To characterize diverse freshwater and marine (estuarine) sediments in and around the continental United States. Sediments were collected from nearly all major physiographic provinces of the U.S. and from a variety of estuarine sites around the U.S. (Figures 2 and 3).

2) To select representative sediments for use in bioavailability studies. Since EPA is initially focusing primarily on neutral organic compounds when developing sediment quality criteria, a neutral organic compound (fluoranthene) was used in this study to amend the sediments.

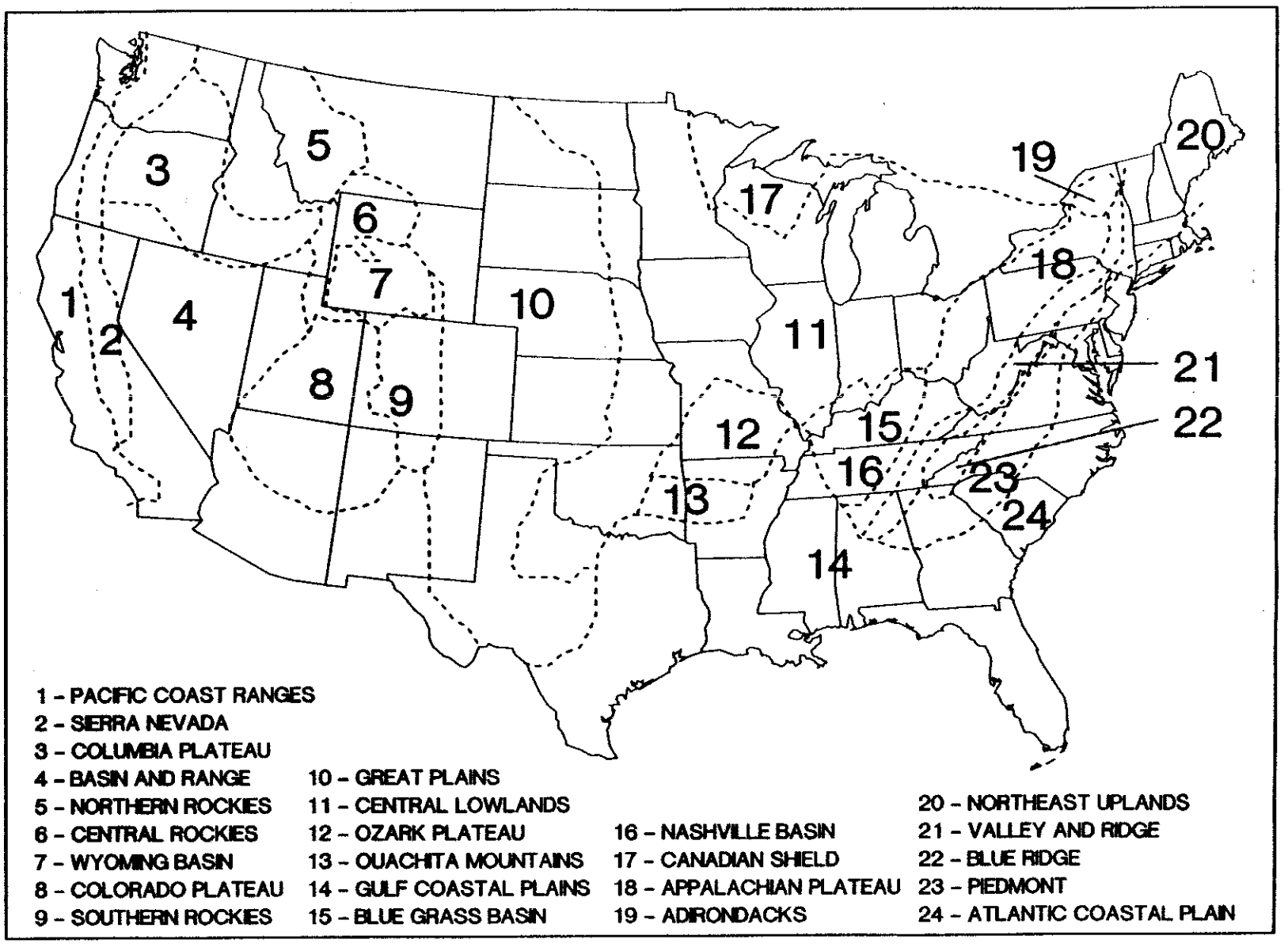


FIGURE 2. Physiographic Provinces of the United States. Within Each Province is a Distinct Uniformity of Geologic Structures, Landforms, and Rock Types That Have Undergone Similar Geologic History (from Government Publications Office, 1987).

FIGURE 3. Sediment Collection Locations. Asterisks Mark Collection Sites.

Hypotheses

1) Sediment characteristics do not differ between physiographic provinces for the parameters analyzed (organic carbon, particle size distribution, particle surface area, cation exchange capacity, redox potential, and percent sediment solids).

2) Sediment characteristics do not differ within physiographic provinces for the parameters analyzed.

3) Marine (estuarine) sediments from various areas on the U.S. coastline do not differ from each other for the parameters analyzed.

4) Freshwater sediments do not differ from marine (estuarine) sediments for the parameters analyzed.

5) There is no significant correlation between sediment toxicity and sediment characteristics (OC, CEC, particle size, surface area).

6) Total organic carbon in sediments does not vary sufficiently enough to be used as a normalizing factor for the bioavailability of potentially toxic materials in sediments.

7) Aquatic organism toxicities due to sediment sorbed fluoranthene do not differ using sediments with similar organic carbon contents.

CHAPTER 2

MATERIALS AND METHODS

Phase I. Sediment Collection and Characterization

This research is concerned with sediments as sources and sinks for contaminants that may be associated with oil spills in fresh and marine waters. Phase I involved the collection and characterization of freshwater and marine sediments from major physiographic provinces (Figure 2) throughout the U.S. The objective was to obtain representative sediments from throughout the U.S. and not necessarily sediments that represented extreme conditions and/or characteristics (Figure 3). Sediments were collected from both pristine sites as well as from urban areas. Sediment collection sites included rivers, streams, lakes, bayous, ocean beaches and bays.

To facilitate sediment sample collection, a shipping package was developed. A postage prepaid watertight shipping container was sent to selected colleagues across the U.S. depending on their location in a given province. Each shipping package contained three or more 700 ml resealable plastic containers, a polypropylene scoop, and a sediment

collection data sheet for each sediment collected (Appendix A).

All sediments were collected in less than one meter of water depth. Once the sediment was collected, it was kept refrigerated or on ice until shipment to University of North Texas (UNT). Sediments were then shipped to UNT on ice via overnight express service. Upon arrival at UNT, sediments were stored at 4°C until analyses were performed. Sediment handling, storage duration and conditions for each parameter followed the recommendations of Plumb (1981) (Table I). This shipping package helped accomplish the objective of sediment collection from diverse sources while at the same time reducing the cost of shipping as well as minimizing sample collection effort.

Sediment characteristics were chosen based on their relative importance as potential normalization factors for the bioavailability of neutral organic compounds sorbed to sediments. Sediment characteristics other than organic carbon content have not received much attention as potential normalization factors. Each sediment parameter and corresponding analysis is listed in Table II and is discussed in much greater detail in Appendix B.

Three sediments were chosen from sediments collected in Phase I for use in bioavailability studies. These sediments are described in detail in Phase II below.

TABLE I. Sediment Storage and Handling for Each Analysis (from Plumb, 1981).

Designation	Cation Exchange Capacity	Particle Size	Redox Potential	Total Organic Carbon	Percent Sediment Solids
Container	PL*	PL	PL	PL	PL
Preservative	4°C	4°C	4°C	4°C	4°C
Storage Time	<1 WK	NC**	ASAP***	NC	NC
Sample Weight Needed (wet)	25g	200g	200g	50g	25g

*PL = Plastic

**NC = Not Critical

***ASAP = As Soon As Possible

Table II. Sediment Parameters and Corresponding Analysis.

Parameter	Analytical Method	Analysis
Organic Carbon	Dohrmann DC-80 Analyzer	Dohrmann, 1985
Cation Exchange Capacity (CEC)	Displacement After Washing	Plumb, 1981
Redox Potential	Corning Redox Probe	Plumb, 1981
Percent Solids	Drying at 104°C	Black, 1986
Particle Size	Hydrometric	Black, 1986
Particle Surface Area	*	Millar, 1965

*Derived from particle size distribution and average particle surface area for each particle size category.

Phase II. Toxicity Testing

Test Organisms

Hyalella azteca was selected for use in this study as a representative macroinvertebrate. Hyalella remains on the sediment surface to feed and hide, thus are in intimate contact with the sediment. Since they do not feed on the sediments, however, they are classified as being intermediate to sediment surface feeders such as daphnids and burrowing benthic organisms such as midges.

Chironomus tentans was selected for testing as a representative sediment dwelling organism. C. tentans is a large midge (second instar 5-10 mm) with a short generation time. It can be cultured in the laboratory and comes in direct contact with the sediment by burrowing into sediment and building a case. Giesy et al. (1988) have successfully used C. tentans in toxicity testing and have shown it to be a sensitive indicator of the presence of bioavailable contaminants associated with sediments.

Daphnia magna was selected for testing as a representative water column organism because of its small size, short life cycle, and ease of culture and handling. Daphnia is also one of the more sensitive aquatic species used in toxicity tests (Lewis and Weber, 1985). Even though daphnids are called water column organisms, they also feedon surface sediments (personal observations). Therefore by

limiting feeding to daphnids during testing, they are forced to feed on the sediments.

Test Sediments

Three test sediments were used during this study. Sediments were selected based on the following criteria: 1) Sediments exhibited no background toxicity in 10 day exposures to the organisms used in this study; 2) Sediments exhibited no detectable background fluoranthene concentrations; 3) Organic carbon contents were similar. The objective was to select three sediments with similar organic carbon contents while varying other sediment characteristics. Characteristics for the three sediments used are listed in Table III.

Toxicity Test Sediments

The three sediments used in this study are briefly described below. All sediments were collected using an Eckman dredge.

1) The Water Research Field Station (WRFS) sediment was collected from the University of North Texas WRFS, Denton County, Texas, in approximately two meters of water.

2) The Trinity River (TR) sediment was collected at river mile 408.5, near Ennis, Texas, on Farm to Market Road 85 in Navarro County.

3) The Lake Fork (LF) sediment was collected from Lake Fork Reservoir in 0.5 meters of water. Lake Fork Reservoir is a

TABLE III. Sediment Characteristics and Corresponding Values for Sediments Used During Phase II (toxicity testing).

Sediment	%OC	CEC (me/100g)	%Sand	%Silt	%Clay	Surface Area (cm^2/g)
Water Research Field Station	0.46	8.82	42.66	55.98	1.36	109,037
Lake Fork Reservior	0.50	3.85	43.96	55.10	0.94	75,710
Trinity River	0.44	11.68	52.75	46.32	0.93	74,881

U.S. Army Corps of Engineers reservoir on Texas State Highway 17 near Quitman, Texas in Wood County.

Test Organism Culture Procedures

Daphnia magna

Daphnia culturing procedures follow the methods described in Biesinger *et al.* (1987), and Peltier and Weber (1984). *Daphnia magna* Strauss cultures were maintained at UNT in light and temperature controlled incubators. *Daphnia* were cultured in 1000 ml beakers containing 800 ml of WRFS pond water (Table IV) with 8 daphnids per beaker. *Daphnia* were fed a synthetic diet daily. *Daphnia* culture methods are described in detail in Appendix C.

Hyalella azteca

Hyalella azteca Saussure (Amphipoda: Crustacea) cultures were maintained at UNT. *Hyalella* were cultured at ambient temperature conditions (~21°C) in 10 gallon glass aquaria in filtered, dechlorinated tap water. Five to eight cm of maple leaves were placed on the bottom of the aquaria for the *Hyalella* to hide in and feed on. Cultures were also fed ground rabbit chow pellets twice per week. *Hyalella* culturing procedures follow the methods described in de March (1981). *Hyalella* culture methods are described in greater detail in Appendix C.

TABLE IV. Water Research Field Station (WRFS) Pond Water Characteristics.

Parameter	Value
pH	7.0 ± 0.5
Dissolved Oxygen	8 - 9 mg/L
Conductivity	350 ± 50 umhos/cm
Alkalinity	165 ± 20 mg/L
Hardness	120 ± 20 mg/L

Chironomus tentans

Chironomus tentans Fabricius (Diptera: Chironomidae) cultures were maintained at UNT. Chironomids were cultured at ambient room temperature in 10 gallon glass aquaria in filtered, dechlorinated tap water. Culture substrate consisted of shredded brown paper towels to a depth of three cm. A suspension of Tetra Conditioning Food was fed to the cultures daily. Chironomid culture methods follow the procedures of Townsend *et al*. (1981). Chironomid culture methods are described in detail in Appendix C.

Testing Procedures

Test Water

The water used in this study was the UNT Water Research Field Station (WRFS) pond water. This pond water is used in our laboratory to culture *Daphnia magna* and has been found to exhibit no toxic effects to the organisms used in this study in 10 day aqueous phase exposures. Pond water characteristics and analyses are listed in Tables IV and V, respectively.

Screening Tests

The first phase of sediment testing involved screening tests to determine whether or not the sediments exhibited any background toxicity to the organisms. *Daphnia*, *Hyalella* and *Chironomus* were exposed to the sediments separately in

TABLE V. Water Analysis Methods and References.

Analysis	Method	Reference*
Temperature	Mercury Thermometer	212
Dissolved Oxygen	YSI Model 54A Meter	421f
pH	Orion Model 250 Meter	423
Conductivity	YSI Model 33 Meter	205.2
Hardness	Titration	314b
Alkalinity	Titration	403

*Standard Methods, 1985

250 ml beakers for 10 days. Each beaker contained 40 ml of sediment and 160 ml of WRFS pond water (4:1 water to sediment ratio). Details of the screening test procedures can be found in Appendix D.

Range - Finding Tests

The second phase of testing involved range-finding tests to determine the range of fluoranthene concentrations needed to cause toxicity to the organisms. There were five treatment concentrations and an untreated control with only one replicate per concentration. Sediments were amended (Appendix E) with varying amounts of fluoranthene usually covering at least one order of magnitude. Test duration was 10 days. Range-finding tests are described in greater detail in Appendix D.

Definitive Tests

The third phase of testing consisted of definitive tests. Sediments were amended with fluoranthene to obtain aqueous concentrations that would span the exposure-response curve for each organism. Definitive test duration was 10 days and consisted of six concentrations and a control with three replicates per concentration. Mortality was defined as immobility by the organisms and therefore effective concentrations (ECs) and not lethal concentrations

(LCs) were used as endpoints. Definitive tests for each organism are described in detail in Appendix D.

Analytical Procedures

Water

Water samples were taken during both range-finding and definitive tests. A 3 ml water sample was taken from each test beaker at the beginning and ending of each test. The sample was collected by pipet, filtered with a Whatman EPM2000 filter, and placed in a 20 ml test tube. Three mls of hexane were then added to the test tube and the mixture subsequently vortex mixed for 30 seconds. The hexane extract was then analyzed on a spectrophotofluorometer (SPF) to determine the free fluoranthene concentration in the water. Water extraction techniques and SPF procedures are given in more detail in Appendix F.

Sediment

Sediment samples were also taken in both the range-finding and definitive tests. One sediment sample was taken from each test beaker at the beginning and ending of each test. Sediment was then extracted twice with 10 ml of hexane, sonicated twice for a total of 6 minutes, and placed in a 20 ml test tube. The hexane extract was analyzed on an SPF to determine the total amount of fluoranthene in the

sediment. Sediment extraction techniques and SPF procedures are given in detail in Appendix F.

Interstitial Water

Interstitial (pore) water samples were collected at the end of each definitive test. Sediment (all 40 ml) from one replicate of each concentration was centrifuged for 10 minutes to obtain the pore water samples. Pore water samples were then extracted with hexane and analyzed on an SPF. Details of the pore water procedures are given in Appendix F.

Statistical Procedures

Differences within and among physiographic provinces along with freshwater versus marine sediments were statistically compared using analysis of variance (SAS, 1985). Correlation analysis (SAS, 1985) was used to examine relationships among sediment characteristics. Correlation analysis was also used in Phase II to determine the relationships between fluoranthene concentrations in sediment and water to organism response (mortality). EC50's for toxicity tests were calculated using the probit procedure from SAS (1985). Mortality response curves were judged to be significantly different if their 95 percent confidence limits did not overlap.

CHAPTER 3

RESULTS

Sediment Characteristics

Tables VI and VII illustrate the results of the freshwater and marine sediment analyses, respectively. Percent organic carbon varied two orders of magnitude and ranged from 0.029 to 11.78% in freshwater sediments and 0.022 to 6.35% in the estuarine sediments. Cation exchange capacity (CEC) varied two orders of magnitude for freshwater sediments (0.09 to 59.17 me/100g) and three orders of magnitude (0.016 to 23.05 me/100g) in marine sediments. Particle surface area varied five orders of magnitude in both freshwater and estuarine sediments. Surface area ranged from 45 to 4,730,533 cm^2/g in freshwater sediments and 45 to 1,958,039 cm^2/g in estuarine sediments. Redox potential ranged from -409 to +379 mv for freshwater sediments and -375 to +321 mv for estuarine sediments indicating that freshwater sediments tended to be more reduced than the estuarine sediment samples.

Physiographic provinces were selected as natural boundaries for dividing the continental U.S. into separate and distinct areas. This division was made in an attempt to determine whether or not SQC could be applied over large

TABLE VI. Range, Mean, Standard Deviation and Number of Replicates for Each Parameter of Freshwater Sediment Samples.

Parameter	Range	Mean	Std Dev.	N
% Solids	11.9 - 91.7	66.3	14.03	308
Redox Potential (mv)	-409 - +379	-45	217.4	302
% Organic* Carbon	0.03 - 11.8	1.36	1.95	308
Cation Exchange Capacity (me/100g)	0.1 - 59.2	5.5	8.27	291
% Sand	2.3 - 100	68.6	29.91	301
% Silt	0 - 94.3	29.5	30.11	301
% Clay	0 - 59.1	3.5	7.51	301
Particle Surface Area (cm^2/g)	45 - 4,730,533	282,238	602,046	300

*Two sites containing coal were deleted because of artificially high organic carbon contents (11.1% and 33.8%).

TABLE VII. Range, Mean, Standard Deviation and Number of Replicates for Each Parameter of Marine and Estuarine Sediment Samples Collected.

Parameter	Range	Mean	Std Dev.	N
% Solids	21.4 - 85.0	67.2	18.40	78
Redox Potential (mv)	-375 - +321	+5	214.0	78
% Organic Carbon	0.02 - 6.35	1.14	1.622	76
Cation Exchange Capacity (me/100g)	0.02 - 23.1	3.6	5.77	78
% Sand	3.2 - 100	79.0	30.22	70
% Silt	0 - 95.1	20.5	29.63	70
% Clay	0 - 24.5	2.5	4.40	70
Particle Surface Area (cm^2/g)	45 - 1,958,039	199,658	352,013	70

areas or if site specific application would be more appropriate. Analysis of variance indicated numerous significant differences within physiographic provinces for all characteristics studied. For example, two samples collected 25 meters apart on the Trinity River, Texas were significantly different ($p<0.0001$). Organic carbon from these two sites varied from 0.50 to 0.88%, and CEC varied from 14.3 to 26.1 me/100g. Three estuarine samples collected within 30 meters of each other on Ocean Isle Beach, North Carolina varied significantly ($p<0.0001$) for both OC and CEC. Organic carbon varied from 0.67 to 3.09%, and CEC varied from 1.57 to 21.80 me/100g.

Analysis of variance indicated significant differences ($p<0.0001$) among provinces for all sediment parameters. Differences between provinces for each parameter are given in Tables VIII - XII. These differences in physical and chemical characteristics suggest that SQC applied on a site specific basis rather than on a broad area basis would be more defensible. Previously, Kimerle (1987) also suggested that SQC would have to be site specific. Percent solids, percent sand, silt, and clay do not vary sufficiently to be used as normalizing factors; however, percent sand, silt and clay could be used to estimate sediment particle surface area quickly and easily as outlined above. Of the sediment characteristics measured, only OC content, CEC and

TABLE VIII. Designated Number, Name, and Number of Sites for Each Province Identified During This Study. Number Designations Refer to Tables IX - XII.

Number Designation	Province Name*	Number of Sites
1	Adirondacks	3
2	Marine - Atlantic Coast	11
3	Appalachian Plateau	3
4	Atlantic Coastal Plain	1
5	Basin and Range	3
6	Blue Ridge	1
7	Canadian Shield	3
8	Colorado Plateau	2
9	Columbia Plateau	3
10	Marine - Gulf Coast	9
11	Gulf Coastal Plains	21
12	Central Lowlands	9
13	Northeast Uplands	6
14	Northern Rockies	3
15	Ouachita Mountains	2
16	Ozark Plateau	4
17	Pacific Coast Ranges	3
18	Piedmont	10
19	Great Plains	5
20	Marine - Pacific Coast	6
21	Central Rockies	3
22	Southern Rockies	5
23	Valley and Ridge	8
24	Wyoming Basin	3
		127

*No samples were collected from Sierra Nevada, Blue Grass Basin or Nashville Basin provinces.

TABLE IX. Table of Probabilities Associated With Percent Sand (Left, Bottom) and Percent Silt (Right, Top) Between Provinces. Number Designations Along the Top and Left Margin are Province Numbers and are Identified in Table VIII. Asterisks Mark Probabilities <0.01.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	.	*	0.24	0.90	0.03	0.53	0.94	*	*	0.19	*	0.38	0.03	0.90	0.49	*	0.01	*	0.90	0.02	0.18	*	*
2	*	.	0.11	0.05	0.85	0.25	*	0.08	*	0.03	0.35	*	0.36	0.09	0.10	0.52	0.50	0.01	*	0.97	0.12	0.24	0.13
3	0.33	0.09	.	0.35	0.28	0.86	0.22	0.01	*	0.93	0.02	0.57	0.43	0.50	0.74	0.07	0.26	*	0.17	0.23	0.93	0.02	0.02
4	0.80	0.05	0.36	.	0.10	0.54	0.94	0.01	*	0.34	0.02	0.50	0.14	0.84	0.53	0.03	0.09	*	0.96	0.08	0.31	0.02	0.01
5	0.03	0.88	0.23	0.09	.	0.35	0.02	0.11	*	0.17	0.44	0.06	0.63	0.16	0.20	0.52	0.80	0.05	0.01	0.91	0.30	0.36	0.14
6	0.50	0.32	0.99	0.46	0.42	.	0.50	0.04	*	0.89	0.13	0.88	0.49	0.69	0.94	0.16	0.38	0.02	0.47	0.31	0.81	0.10	0.05
7	0.86	*	0.26	0.90	0.02	0.43	.	*	*	0.17	*	0.35	0.03	0.86	0.45	*	0.01	*	0.97	0.02	0.17	*	*
8	*	0.07	0.01	*	0.10	0.05	*	.	0.12	*	0.18	*	0.03	0.01	0.01	0.26	0.03	0.96	*	0.13	0.01	0.28	0.85
9	*	*	*	*	*	*	*	0.14	.	*	*	*	*	*	*	*	*	0.04	*	*	*	*	0.31
10	0.24	0.03	0.98	0.32	0.17	0.99	0.18	*	*	.	*	0.52	0.27	0.50	0.76	0.02	0.10	*	0.11	0.13	0.84	*	0.01
11	*	0.12	0.01	0.01	0.28	0.12	*	0.27	*	*	.	*	0.07	0.04	0.03	0.96	0.08	0.05	*	0.54	0.02	0.67	0.23
12	0.39	*	0.73	0.43	0.08	0.83	0.30	*	*	0.63	*	.	0.08	0.70	0.93	*	0.01	*	0.26	0.05	0.47	*	0.06
13	0.07	0.27	0.47	0.15	0.51	0.66	0.05	0.02	*	0.37	0.01	0.17	.	0.23	0.31	0.19	0.72	*	0.01	0.54	0.47	0.06	0.06
14	0.60	0.25	0.89	0.53	0.34	0.90	0.52	0.04	*	0.87	0.08	0.95	0.55	.	0.70	0.06	0.16	0.01	0.83	0.13	0.45	0.03	0.02
15	0.20	0.39	0.67	0.25	0.53	0.77	0.16	0.04	*	0.64	0.09	0.44	0.89	0.66	.	0.06	0.19	*	0.41	0.17	0.68	0.02	0.02
16	*	0.42	0.04	0.02	0.46	0.18	*	0.29	*	0.01	0.85	*	0.11	0.14	0.19	.	0.26	0.16	*	0.60	0.07	0.81	0.28
17	0.01	0.53	0.21	0.08	0.80	0.46	0.01	0.03	*	0.09	0.02	0.02	0.56	0.37	0.60	0.21	.	*	*	0.69	0.27	0.07	0.07
18	*	0.01	*	*	0.04	0.03	*	0.97	0.07	*	0.08	*	*	0.02	0.01	0.15	*	.	*	0.07	*	0.15	0.79
19	0.78	*	0.19	0.94	0.01	0.38	0.94	*	*	0.10	*	0.20	0.02	0.47	0.12	*	*	*	.	0.01	0.12	*	*
20	0.02	0.96	0.18	0.07	0.88	0.36	0.02	0.14	*	0.17	0.38	0.05	0.40	0.29	0.45	0.57	0.65	0.06	0.01	.	0.24	0.41	0.16
21	0.20	0.14	0.80	0.27	0.32	0.88	0.16	0.01	*	0.78	0.01	0.50	0.63	0.76	0.82	0.06	0.31	*	0.10	0.24	.	0.02	0.03
22	*	0.25	0.01	0.01	0.36	0.14	*	0.26	*	*	0.87	*	0.04	0.10	0.13	0.95	0.08	0.09	*	0.47	0.02	.	0.31
23	*	0.11	0.02	0.01	0.12	0.06	*	0.80	0.37	0.01	0.27	0.01	0.04	0.04	0.05	0.27	0.06	0.80	*	0.15	0.02	0.26	.

TABLE X. Table of Probabilities Associated With Percent Clay (Left, Bottom) and Surface Area (Right, Top) Between Provinces. Number Designations Along the Top and Left Margin are Province Numbers and are Identified in Table VIII. Asterisks Mark Probabilities <0.01.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	.	0.02	0.03	0.07	0.28	0.31	0.03	0.05	0.01	0.03	0.20	0.23	0.01	0.52	*	0.05	*	0.05	0.25	0.03	0.78	0.01	0.20
2	0.02	.	0.64	0.52	0.39	0.80	0.59	0.63	0.30	0.97	0.07	0.14	0.55	0.04	*	0.98	0.30	0.96	0.31	0.68	0.01	0.61	0.97
3	0.03	0.64	.	0.74	0.29	0.62	0.94	0.93	0.64	0.67	0.10	0.14	1.00	0.03	*	0.71	0.82	0.65	0.23	0.98	0.01	0.92	0.82
4	0.07	0.52	0.74	.	0.29	0.51	0.78	0.81	1.00	0.54	0.19	0.21	0.73	0.05	*	0.56	0.82	0.52	0.26	0.73	0.04	0.67	0.66
5	0.28	0.39	0.29	0.29	.	0.80	0.27	0.31	0.13	0.40	0.86	0.88	0.23	0.17	*	0.45	0.13	0.46	0.97	0.31	0.16	0.24	0.61
6	0.31	0.80	0.62	0.51	0.80	.	0.59	0.60	0.42	0.79	0.70	0.71	0.60	0.19	*	0.80	0.50	0.82	0.78	0.64	0.23	0.64	0.83
7	0.03	0.59	0.94	0.78	0.27	0.59	.	0.99	0.70	0.62	0.10	0.13	0.93	0.03	*	0.66	0.90	0.60	0.22	0.92	0.01	0.85	0.79
8	0.05	0.63	0.93	0.81	0.31	0.60	0.99	.	0.74	0.66	0.16	0.19	0.93	0.04	*	0.69	0.93	0.64	0.27	0.91	0.02	0.86	0.79
9	0.01	0.30	0.64	1.00	0.13	0.42	0.70	0.74	.	0.34	0.03	0.05	0.59	0.02	*	0.39	0.72	0.34	0.10	0.62	*	0.50	0.59
10	0.03	0.97	0.67	0.54	0.40	0.79	0.62	0.66	0.34	.	0.10	0.16	0.60	0.04	*	1.00	0.36	0.94	0.32	0.71	0.01	0.66	0.98
11	0.20	0.07	0.11	0.19	0.86	0.70	0.10	0.16	0.03	0.10	.	0.98	0.03	0.15	*	0.21	*	0.19	0.87	0.13	0.08	0.02	0.49
12	0.23	0.14	0.14	0.21	0.88	0.71	0.13	0.19	0.05	0.16	0.98	.	0.06	0.16	*	0.26	0.01	0.24	0.90	0.16	0.10	0.05	0.51
13	0.01	0.55	1.00	0.73	0.23	0.60	0.94	0.93	0.59	0.60	0.03	0.06	.	0.02	*	0.66	0.78	0.59	0.16	0.97	*	0.89	0.81
14	0.52	0.04	0.03	0.05	0.17	0.19	0.03	0.04	0.02	0.04	0.15	0.16	0.02	.	*	0.05	0.01	0.05	0.16	0.04	0.64	0.02	0.12
15	*	*	*	*	*	*	*	*	*	*	*	*	*	*	.	*	*	*	*	*	*	*	*
16	0.05	0.98	0.71	0.56	0.45	0.80	0.66	0.68	0.39	1.00	0.21	0.26	0.66	0.05	*	.	0.47	0.95	0.39	0.74	0.02	0.72	0.98
17	*	0.30	0.82	0.82	0.13	0.50	0.90	0.93	0.72	0.36	*	0.01	0.78	0.01	*	0.47	.	0.38	0.07	0.80	*	0.63	0.70
18	0.05	0.96	0.65	0.52	0.46	0.82	0.60	0.64	0.34	0.94	0.19	0.24	0.59	0.05	*	0.95	0.38	.	0.39	0.68	0.02	0.64	0.95
19	0.25	0.31	0.23	0.26	0.97	0.78	0.22	0.27	0.10	0.32	0.87	0.90	0.16	0.16	*	0.39	0.07	0.39	.	0.26	0.13	0.16	0.58
20	0.03	0.68	0.98	0.73	0.31	0.64	0.92	0.91	0.62	0.71	0.13	0.16	0.97	0.04	*	0.74	0.80	0.68	0.26	.	0.01	0.95	0.84
21	0.78	0.01	0.01	0.04	0.16	0.23	0.01	0.02	*	0.01	0.08	0.10	*	0.64	*	0.02	*	0.02	0.13	0.01	.	*	0.14
22	0.01	0.61	0.92	0.67	0.24	0.64	0.85	0.86	0.50	0.66	0.02	0.05	0.89	0.02	*	0.72	0.63	0.64	0.16	0.95	*	.	0.86
23	0.20	0.97	0.82	0.66	0.61	0.83	0.79	0.79	0.59	0.98	0.49	0.51	0.81	0.12	*	0.98	0.70	0.95	0.58	0.84	0.14	0.86	.

TABLE XI. Table of Probabilities Associated With Percent Organic Carbon (Left, Bottom) and Cation Exchange Capacity (Right, Top) Between Provinces. Number Designations Along the Top and Left Margin are Province Numbers and are Identified in Table VIII. Asterisks Mark Probabilities <0.01.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	.	0.02	0.64	0.93	0.06	0.98	0.96	*	*	0.62	0.04	0.37	0.03	0.08	0.59	*	0.16	*	0.97	0.88	0.13	0.48	0.05
2	0.03	.	0.09	0.13	0.94	0.15	0.02	*	0.03	0.01	0.55	0.05	0.86	0.95	0.20	0.22	0.20	0.19	*	0.04	0.47	0.03	0.72
3	0.15	0.67	.	0.67	0.16	0.72	0.60	*	*	0.94	0.15	0.75	0.09	0.19	0.90	0.02	0.41	0.01	0.56	0.75	0.32	0.90	0.13
4	0.64	0.07	0.15	.	0.15	0.96	0.96	*	0.01	0.68	0.18	0.51	0.12	0.18	0.62	0.04	0.32	0.04	0.94	0.84	0.27	0.59	0.13
5	0.54	0.17	0.44	0.38	.	0.17	0.05	*	0.08	0.07	0.66	0.16	0.96	0.91	0.25	0.38	0.35	0.36	0.03	0.08	0.55	0.11	0.81
6	0.94	0.16	0.30	0.75	0.62	.	0.99	*	0.01	0.73	0.20	0.55	0.14	0.20	0.67	0.05	0.36	0.04	1.00	0.89	0.30	0.63	0.14
7	0.96	0.03	0.15	0.67	0.52	0.97	.	*	*	0.57	0.03	0.33	0.02	0.07	0.55	*	0.14	*	0.98	0.83	0.12	0.44	0.05
8	0.03	0.47	0.36	0.04	0.11	0.10	0.03	.	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
9	0.01	0.30	0.24	0.03	0.06	0.07	0.01	0.90	.	*	0.01	*	0.05	0.07	0.01	0.33	*	0.30	*	*	0.01	*	0.19
10	0.68	0.01	0.19	0.45	0.74	0.74	0.65	0.03	0.01	.	0.02	0.57	0.02	0.09	0.83	*	0.19	*	0.50	0.76	0.16	0.77	0.07
11	0.96	*	0.06	0.62	0.40	0.96	0.99	0.01	*	0.46	.	0.09	0.51	0.77	0.29	0.09	0.38	0.06	0.01	0.06	0.74	0.06	0.50
12	0.74	*	0.04	0.76	0.29	0.90	0.79	0.01	*	0.30	0.63	.	0.06	0.20	0.90	0.01	0.47	*	0.23	0.48	0.36	0.80	0.14
13	0.02	0.74	0.52	0.05	0.13	0.13	0.02	0.63	0.47	0.01	*	*	.	0.85	0.18	0.33	0.21	0.30	0.01	0.04	0.43	0.04	0.82
14	*	*	*	*	*	*	*	0.01	0.01	*	*	*	*	.	0.30	0.32	0.43	0.30	0.04	0.11	0.64	0.15	0.73
15	0.79	0.04	0.13	0.81	0.43	0.90	0.83	0.03	0.01	0.52	0.78	0.98	0.03	*	.	0.05	0.57	0.04	0.52	0.69	0.46	0.98	0.20
16	0.06	0.95	0.68	0.08	0.23	0.18	0.06	0.54	0.40	0.06	0.01	0.01	0.83	*	0.06	.	0.03	1.00	*	0.01	0.10	*	0.61
17	0.72	0.01	0.07	0.82	0.33	0.86	0.76	0.01	*	0.39	0.67	0.92	0.01	*	0.96	0.02	.	0.02	0.06	0.22	0.75	0.33	0.28
18	0.05	0.69	0.87	0.10	0.27	0.22	0.06	0.34	0.19	0.04	*	*	0.50	*	0.06	0.73	0.02	.	*	*	0.08	*	0.60
19	0.62	0.06	0.29	0.42	0.85	0.69	0.59	0.06	0.02	0.88	0.45	0.31	0.05	*	0.48	0.12	0.37	0.11	.	0.83	0.06	0.35	0.03
20	0.68	*	0.05	0.82	0.28	0.85	0.73	0.01	*	0.31	0.59	0.90	*	*	0.95	0.01	1.00	*	0.31	.	0.18	0.61	0.07
21	0.85	0.02	0.11	0.74	0.43	0.95	0.89	0.02	0.01	0.53	0.84	0.93	0.02	*	0.93	0.04	0.87	0.04	0.49	0.85	.	0.27	0.43
22	*	0.05	0.06	0.01	0.01	0.02	*	0.55	0.60	*	*	*	0.14	0.02	*	0.13	*	0.02	*	*	*	.	0.10
23	*	*	*	*	*	*	*	0.17	0.16	*	*	*	0.01	0.10	*	0.01	*	*	*	*	*	0.30	.
24	0.08	0.97	0.75	0.10	0.28	0.21	0.08	0.51	0.38	0.09	0.02	0.01	0.78	*	0.08	0.94	0.04	0.82	0.16	0.02	0.06	0.13	0.01

TABLE XII. Table of Probabilities Associated With Redox Potential (Left, Bottom) and Percent Solids (Right, Top) Between Provinces. Number Designations Along the Top and Left Margin are Province Numbers and are Identified in Table VIII. Asterisks Mark Probabilities <0.01.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	.	*	0.81	0.69	0.19	0.96	0.99	*	*	0.47	0.13	0.80	0.01	*	0.83	0.06	0.94	0.02	0.01	0.56	0.46	*	0.01
2	0.16	.	*	*	0.02	0.01	*	0.19	0.03	*	*	*	0.24	*	*	0.06	*	0.02	0.19	*	*	0.40	0.10
3	0.63	0.43	.	0.82	0.12	0.90	0.83	*	*	0.31	0.07	0.59	*	*	1.00	0.03	0.87	0.01	0.01	0.76	0.33	*	*
4	0.73	0.21	0.49	.	0.19	0.77	0.70	*	*	0.37	0.17	0.55	0.03	*	0.83	0.09	0.73	0.05	0.04	0.96	0.36	0.02	0.03
5	0.47	0.02	0.22	0.87	.	0.33	0.20	0.01	*	0.38	0.83	0.17	0.21	*	0.17	0.61	0.17	0.45	0.29	0.04	0.57	0.15	0.28
6	*	*	*	*	*	.	0.97	*	*	0.61	0.33	0.84	0.06	*	0.91	0.18	0.99	0.12	0.08	0.74	0.57	0.05	0.08
7	0.33	0.01	0.14	0.73	0.80	*	.	*	*	0.48	0.14	0.80	0.01	*	0.84	0.06	0.95	0.02	0.01	0.59	0.46	0.01	0.01
8	0.03	0.15	0.08	0.05	*	0.02	*	.	0.62	*	*	*	0.05	0.29	*	0.02	*	0.01	0.04	*	*	0.08	0.03
9	*	*	*	*	*	0.93	*	*	.	*	*	*	*	0.53	*	*	*	*	*	*	*	0.01	*
10	*	*	*	*	*	0.04	*	0.34	*	.	0.25	0.50	0.01	*	0.39	0.10	0.41	0.02	0.01	0.09	0.85	*	0.01
11	*	*	*	*	*	0.03	*	0.40	*	0.76	.	0.05	0.03	*	0.13	0.33	0.10	0.09	0.06	*	0.59	0.02	0.04
12	0.04	0.34	0.16	0.10	*	*	*	0.39	*	*	*	.	*	*	0.64	0.03	0.73	*	*	0.27	0.51	*	*
13	0.24	0.89	0.53	0.25	0.04	*	0.02	0.15	*	*	*	0.34	.	*	0.01	0.43	*	0.44	0.85	*	0.05	0.81	0.76
14	0.52	0.55	0.88	0.42	0.17	*	0.11	0.10	*	*	*	0.22	0.66	.	*	*	*	*	*	*	*	*	*
15	0.35	0.02	0.17	0.71	0.77	*	0.95	*	*	*	*	0.01	0.04	0.13	.	0.06	0.89	0.02	0.01	0.79	0.38	0.01	0.01
16	0.84	0.07	0.47	0.83	0.57	*	0.40	0.01	*	*	*	0.01	0.12	0.37	0.42	.	0.05	0.85	0.56	*	0.26	0.33	0.58
17	*	*	*	0.08	0.02	*	0.04	*	*	*	*	*	*	*	0.08	*	.	0.01	0.01	0.63	0.41	*	0.01
18	0.33	0.53	0.72	0.32	0.06	*	0.03	0.08	*	*	*	0.12	0.69	0.86	0.05	0.18	*	.	0.61	*	0.14	0.32	0.63
19	0.05	0.36	0.17	0.10	0.01	*	*	0.45	*	0.01	0.01	0.93	0.36	0.23	0.01	0.02	*	0.16	.	*	0.09	0.68	0.93
20	0.03	*	0.01	0.28	0.17	*	0.28	*	*	*	*	*	*	*	0.39	0.03	0.20	*	*	.	0.15	*	*
21	*	*	*	*	*	0.12	*	0.21	0.02	0.55	0.40	0.01	*	*	*	*	*	*	0.01	*	.	0.04	0.08
22	*	0.01	0.01	0.01	*	0.02	*	0.66	*	0.50	0.61	0.06	0.01	0.01	*	*	*	*	0.11	*	0.29	.	0.58
23	*	*	*	*	*	0.14	*	0.09	0.01	0.21	0.08	*	*	*	*	*	*	*	*	*	0.76	0.08	.
24	0.04	0.22	0.11	0.07	0.01	0.01	*	0.87	*	0.24	0.29	0.52	0.22	0.15	0.01	0.02	*	0.12	0.58	*	0.15	0.52	0.06

particle surface area varied at least two orders of magnitude. This range is sufficiently broad to be examined as potential normalization factors for use in bioavailability estimation. Correlation analysis was performed to determine the relationships between the measured sediment characteristics (Table XIII). Percent OC was significantly correlated with CEC and percent silt but negatively correlated with percent sand, clay and surface area. Cation exchange capacity, like OC, was also significantly correlated with percent silt and negatively correlated with percent sand, clay and surface area. Since surface area was calculated from particle size distribution, it is therefore driven largely by percent clay and hence highly correlated to percent clay.

Freshwater sediment parameter values were comparable to estuarine and marine sediment parameter values (Tables VI and VII). Organic carbon content of freshwater sediments (1.36%) was slightly higher than that of estuarine sediments (1.14%). Cation exchange capacity was slightly higher in freshwater sediments (5.5 me/100g) as compared to estuarine sediments (3.6 me/100g).

Toxicity Testing

In preliminary aqueous phase 10 day exposures C. tentans (EC50 = 31.9 ug/l) and H. azteca (EC50 = 44.9 ug/l) (Table XIV) responded similarly to fluoranthene and

TABLE XIII. Pearson Correlation Coefficients and Number of Replicates (N) for Each Coefficient Listed. All Coefficients are Significant at p = 0.05.

	Solids	OC	CEC	Sand	Silt	Clay
OC	-0.721 (368)					
CEC	-0.613 (367)	0.378 (358)				
Sand	0.721 (347)	-0.473 (349)	-0.646 (345)			
Silt	-0.702 (347)	0.480 (349)	0.648 (345)	-0.992 (370)		
Clay	0.181 (347)	-0.156 (349)	-0.136 (345)	0.161 (370)	-0.221 (370)	
Surface Area	0.181 (347)	-0.156 (349)	-0.136 (370)	0.161 (370)	-0.221 (370)	1.000 (370)

TABLE XIV. Comparison of EC50 Values (95% C.I.) Between 10 Day Aqueous and Whole Sediment Tests for Each Organism Studied. Aqueous Phase EC50's are Initial Concentrations. Water and Pore Water Concentrations are in ug/L. Sediment Concentrations are in mg/kg Dry Weight.

	D. magna	*H. azteca*	*C. tentans*
Aqueous Phase Tests			
	102.6(96.3-110.1)	44.9(32.8-66.5)	31.9(15.2-94.5)
Whole Sediment Tests			
Ending Water			
WRFS	91.6(81.6-97.1)	44.7(36.6-53.9)	61.0(49.2-71.8)
LF	64.1(52.2-70.4)	54.0(47.1-63.2)	50.6(44.0-59.1)
TR	42.7(23.1-55.5)	32.4(28.4-37.2)	30.4(25.6-36.1)
Pore Water			
WRFS	158.0(140.3-170.0)	45.9(38.5-55.0)	91.2(72.8-109.0)
LF	197.3(175.3-215.8)	236.5(185.3-342.2)	251.0(240.7-267.3)*
TR	88.6(33.2-120.9)	97.6(77.8-122.1)	75.7(65.0-88.4)
Ending Sediment			
WRFS	15.0(6.9-19.2)	2.33(1.62-3.40)	7.26(5.13-9.77)
LF	11.9(9.9-13.5)	7.37(6.31-8.82)	8.71(7.36-10.52)
TR	4.2(0.7-7.3)	5.52(4.78-6.42)	2.96(2.42-3.66)

*EC50 value and 95% C.I. were calculated using the computer program developed by Stephan (1977).

WRFS = Water Research Field Station
LF = Lake Fork Reservoir
TR = Trinity River

are illustrated in Figure 4. However, three day old D. magna were less sensitive to fluoranthene (EC50 = 102.6 ug/l) than either Hyalella or Chironomus.

Tables XV - XXIII present measured fluoranthene concentrations in water, interstitial (pore) water and sediment for all definitive tests conducted. Ending pore water concentrations in Trinity River (TR) sediment tests were two to six times greater than ending water concentrations. Lake Fork (LF) sediment tests had pore water concentrations four to ten times higher than ending water concentrations at the lowest fluoranthene concentrations. However, Water Research Field Station (WRFS) sediment tests had pore water concentrations that were within a factor of two of the water concentrations at the end of the tests for all but the lowest fluoranthene concentrations. These results demonstrate that fluoranthene concentrations in overlying water may be only a fraction of pore water concentrations and may vary up to an order of magnitude. At lower sediment concentrations (<8500 ug/kg), pore water fluoranthene concentrations were two to ten times the overlying water concentrations.

Ending sediment concentrations were two to six times lower than beginning sediment concentrations in all tests (Tables XV - XXIII). Fluoranthene recovery from sediment was dependent on the amount of fluoranthene added to the

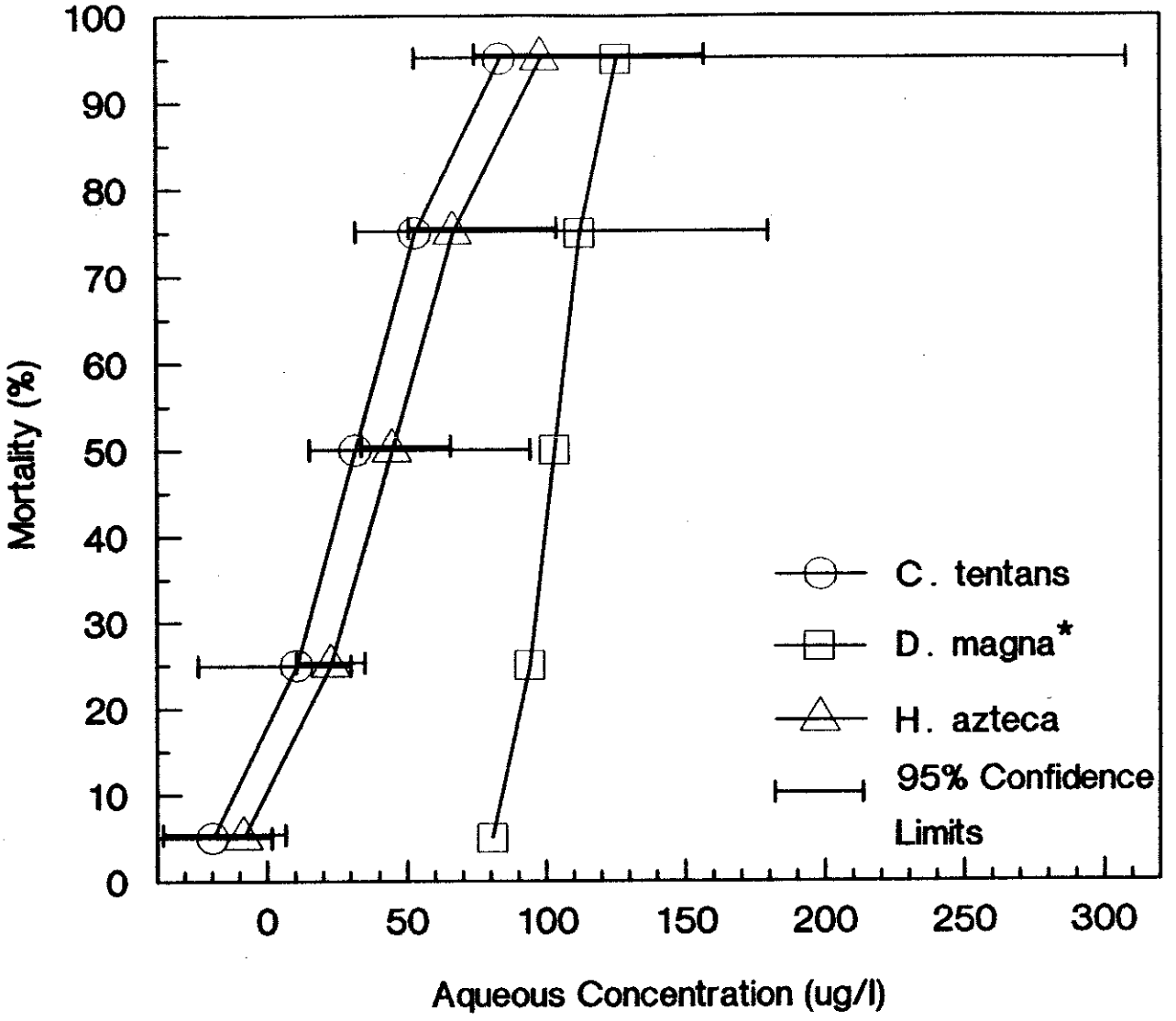


FIGURE 4. Mortality Response of Daphnia magna, Hyalella azteca, and Chironomus tentans Versus Fluoranthene Concentration in Water. Asterisk Indicates that 95% Confidence Limits could not be Calculated for this Mortality Response Relationship.

TABLE XV. Effects of Fluoranthene on C. tentans Using Water Research Field Station Sediment.

Fluoranthene added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	100	-	0.00	0.00	0.00	0.00
100	93	21.5	5.0	2020	373	21.3
500	73	45.3	36.0	9048	1419	42.0
1000	33	77.3	73.3	17941	4790	98.0
2000	27	110.0	105.0	37679	16855	162.1
2500	6.7	121.3	107.3	47317	18629	162.1
3500	0.0	118.7	102.7	69969	28742	182.1

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XVI. Effects of Fluoranthene on C. tentans Using Lake Fork Reservoir Sediment.

Fluoranthene Added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	93	0.0	0.0	0.0	0.0	0.0
250	100	6.67	6.22	5097	1135	56.03
500	100	13.34	14.23	9842	1856	88.04
750	93	22.23	26.01	14846	2238	140.07
1000	67	32.46	44.47	20688	6019	264.13
1250	47	44.24	57.58	25996	11098	272.14
1500	13	56.69	61.36	29437	10721	248.12

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XVII. Effects of Fluoranthene on C. tentans Using Trinity River Sediment.

Fluoranthene Added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	87	0.0	0.0	0.0	0.0	0.0
300	80	5.34	14.01	5846	1122	34.68
450	93	6.67	19.12	8840	1455	45.36
600	80	11.34	26.90	12299	2501	64.03
750	27	15.56	37.35	16364	3687	86.04
900	6.7	19.79	49.14	19374	5016	130.07
1150	0.0	38.91	66.03	25581	8464	144.07

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XVIII. Effects of Fluoranthene on H. azteca Using Water Research Field Station Sediment.

Fluoranthene Added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	100	0.00	0.00	0.00	0.00	0.00
100	93	19.8	5.33	2094	408	14.0
500	47	78.0	43.0	10941	1987	44.0
900	3.3	127.3	104.0	16826	6091	94.0
1300	0.0	102.7	108.7	23427	7038	146.1
1700	0.0	206.7	123.3	32387	13307	156.1
2100	0.0	153.3	125.3	42108	18275	162.1

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XIX. Effects of Fluoranthene on H. azteca Using Lake Fork Reservoir Sediment.

Fluoranthene Added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	90	0.0	0.0	0.0	0.0	0.0
100	97	5.34	1.11	2062	495	20.01
400	93	13.34	7.34	7813	1229	54.69
700	90	25.57	23.34	13663	2464	136.07
1000	73	40.02	39.35	20565	4195	200.10
1300	40	48.92	59.36	25706	7022	232.12
1600	30	57.81	68.70	33211	10784	284.14

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XX. Effects of Fluoranthene on H. azteca Using Trinity River Sediment.

Fluoranthene Added to Sediment ug/kg	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	97	0.0	0.0	0.0	0.0	0.0
300	90	6.22	6.45	6334	1241	32.68
500	73	14.23	17.34	8658	2088	60.03
700	80	19.79	24.23	13552	2787	82.04
900	67	26.46	25.57	18230	5154	96.05
1100	13	25.12	52.47	21962	9467	142.07
1300	0.0	45.13	70.70	25547	11662	152.08

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XXI. Effects of Fluoranthene on D. magna Using Water Research Field Station Sediment.

Fluoranthene Added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	100	0.0	0.0	0.0	0.0	0.0
900	100	22.01	62.03	20758	4909	104.05
1500	20	43.58	98.71	35130	16678	176.09
1700	40	44.47	102.72	40985	19339	156.08
1900	13	39.80	101.38	42226	19872	193.43
2100	27	43.80	110.05	44533	22710	206.77
2300	0.0	51.14	116.73	48614	26081	226.78

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XXII. Effects of Fluoranthene on D. magna Using Lake Fork Reservoir Sediment.

Fluoranthene Added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	100	0.0	0.0	0.0	0.0	0.0
800	93	36.46	35.80	15904	4646	121.43
1200	87	57.14	58.70	23993	9342	143.40
1500	47	70.71	65.36	30144	12790	249.00
1700	6.7	73.37	72.04	37054	15549	231.23
1900	27	68.70	76.71	33752	14295	271.23
2100	0.0	89.38	79.37	43675	21004	255.67

[a]All sediment concentrations expressed on a dry weight basis.

TABLE XXIII. Effects of Fluoranthene on D. magna Using Trinity River Sediment.

Fluoranthene Added to Sediment ug/kg[a]	Percent Survival	Beginning Water Conc. ug/L	Ending Water Conc. ug/L	Beginning Sediment Conc. ug/kg	Ending Sediment Conc. ug/kg	Ending Pore Water Conc. ug/L
Control	100	0.0	0.0	0.0	0.0	0.0
500	87	35.13	22.01	9167	2219	48.69
1000	6.7	72.04	55.36	18183	3894	106.05
1300	27	86.04	70.04	25316	8715	144.07
1500	0.0	87.38	82.04	28487	11348	170.09
1700	0.0	84.71	84.04	32867	16427	176.09
1900	0.0	100.72	90.05	38138	18433	192.10

[a]All sediment concentrations expressed on a dry weight basis.

sediments with maximum recovery of fluoranthene occurring in highest sediment fluoranthene concentrations.

Behavioral and Other Subjective Observations

Control mortality in all tests did not exceed 10% except in the C. tentans TR test where control mortality was 13%. Midges in controls and fluoranthene concentrations in overlying water less than forty ug/l had buried themselves and constructed cases within 48 h of test initiation. However, midges exposed to overlying water fluoranthene concentrations in excess of forty ug/l were unable to bury into the sediment or construct cases and subsequently died. This behavior (failure to bury themselves) was observed in all midge tests. C. tentans test results are presented in Tables XV - XVII.

Hyalella swam to the sediment surface upon placement in test vessels and could not be seen thereafter consequently no sublethal effects could be observed. Upon death, however, Hyalella became whitish in color and mortality could easily be monitored. H. azteca test results are presented in Tables XVIII - XX.

Daphnia in control beakers and fluoranthene concentrations in overlying water of less than 35 ug/l fed more frequently from the sediment surface than daphnids exposed to sediments with overlying water concentrations

greater than 35 ug/l. Sediment was also noted in greater quantities in daphnid guts in controls. This sediment avoidance behavior was noted in all sediment tests spiked with fluoranthene. Increased sediment surface feeding behavior resulted in higher suspended solids concentrations in control beakers. After a few days, 'flea prints' were noted on sediments where the daphnids had previously fed on the sediment. Very few, if any, 'flea prints' were observed on sediments with overlying water concentrations in excess of 35 ug/l. D. magna test results are presented in Tables XXI - XXIII.

Figures 5 - 13 illustrate the exposure response relationships of C. tentans, H. azteca and D. magna to fluoranthene concentrations in overlying water, pore water and sediment for the three sediments examined in this study. Significant differences were found for each of the three organisms based on overlying water, pore water and sediment fluoranthene concentrations. Overlying water, pore water and sediment fluoranthene concentrations do not appear to be accurate predictors of bioavailability. Ten day EC50 values for water, pore water and sediment (Table XIV) varied by up to a factor of four. Since organic carbon was held constant, organic carbon does not appear to be the only factor affecting bioavailability of fluoranthene (a neutral organic compound) to the organisms tested.

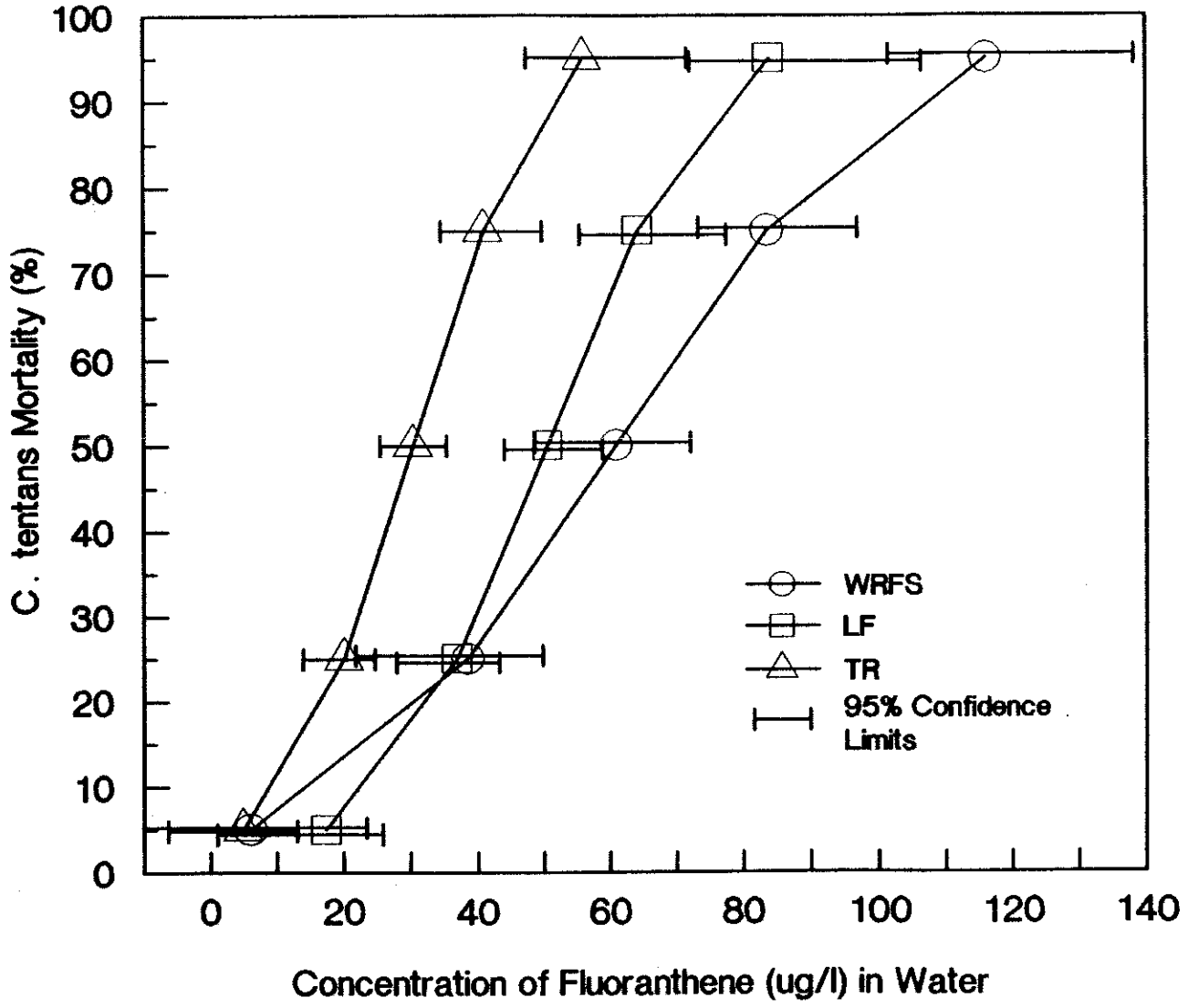


FIGURE 5. Mortality Response of Chironomus tentans Versus Fluoranthene Concentration in Overlying Water.

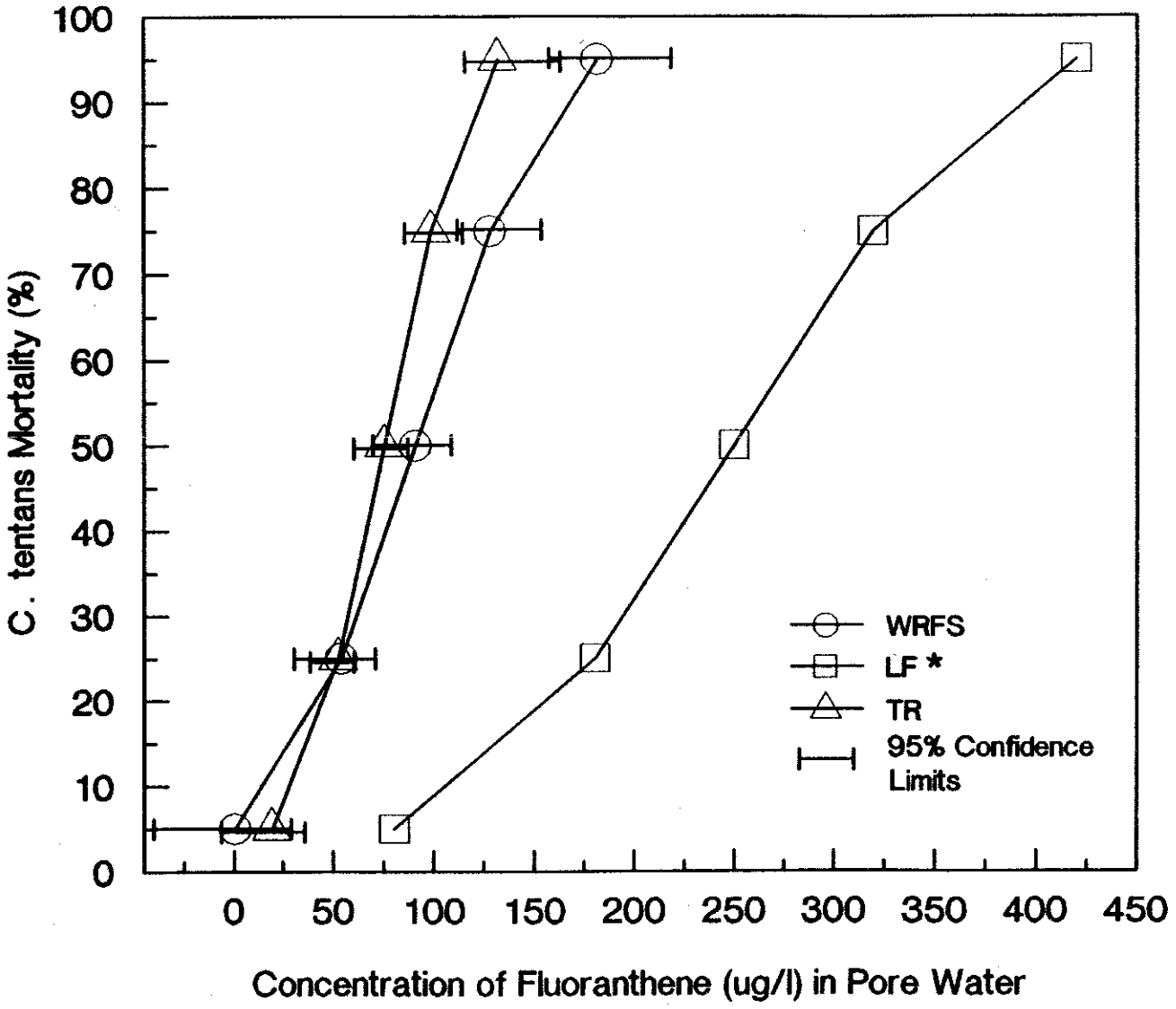


FIGURE 6. Mortality Response of Chironomus tentans Versus Fluoranthene Concentration in Pore Water. Asterisk Indicates that 95% Confidence Limits could not be Calculated for this Mortality Response Relationship.

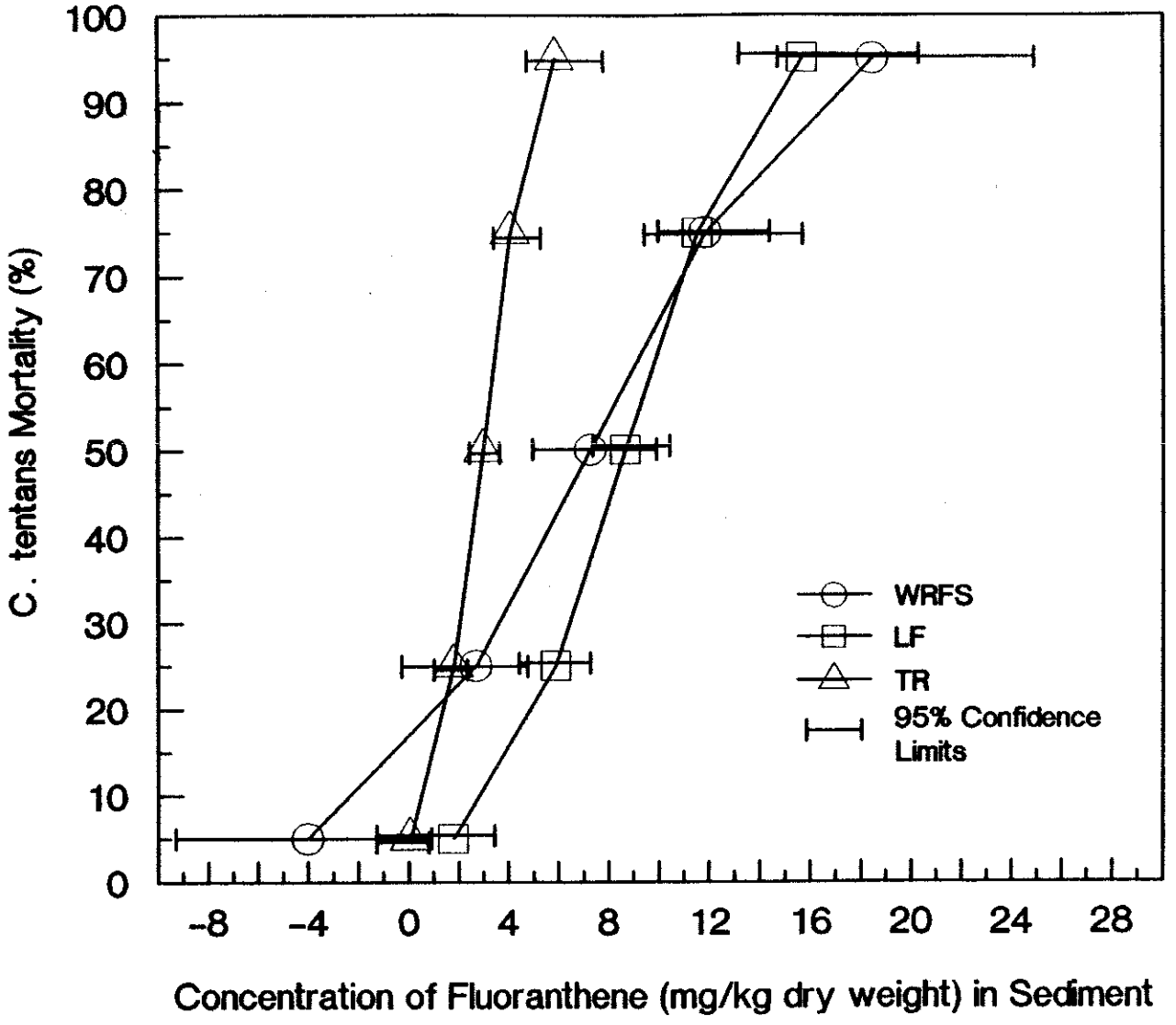


FIGURE 7. Mortality Response of Chironomus tentans Versus Fluoranthene Concentration in Sediment.

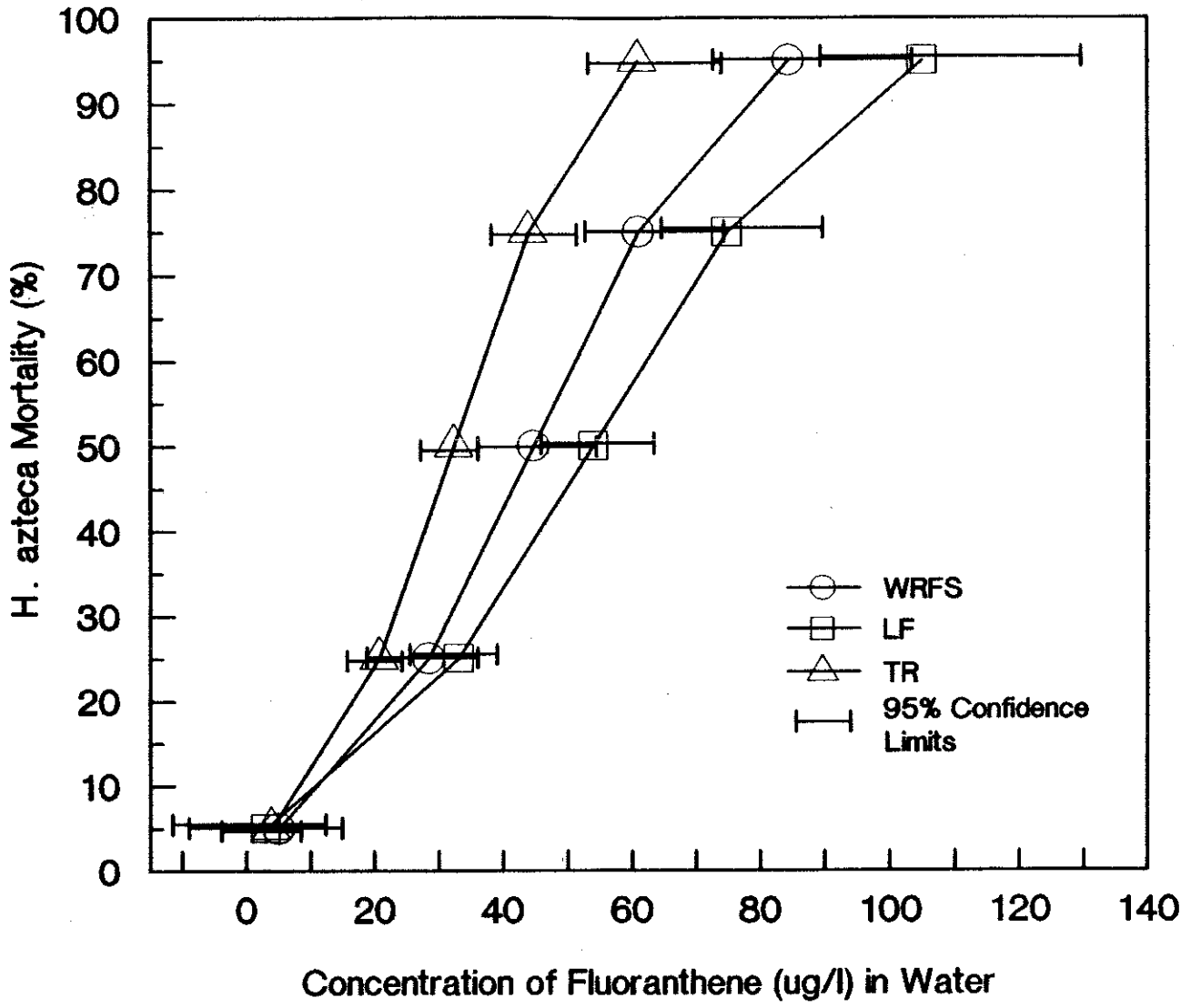


FIGURE 8. Mortality Response of Hyalella azteca Versus Fluoranthene Concentration in Overlying Water.

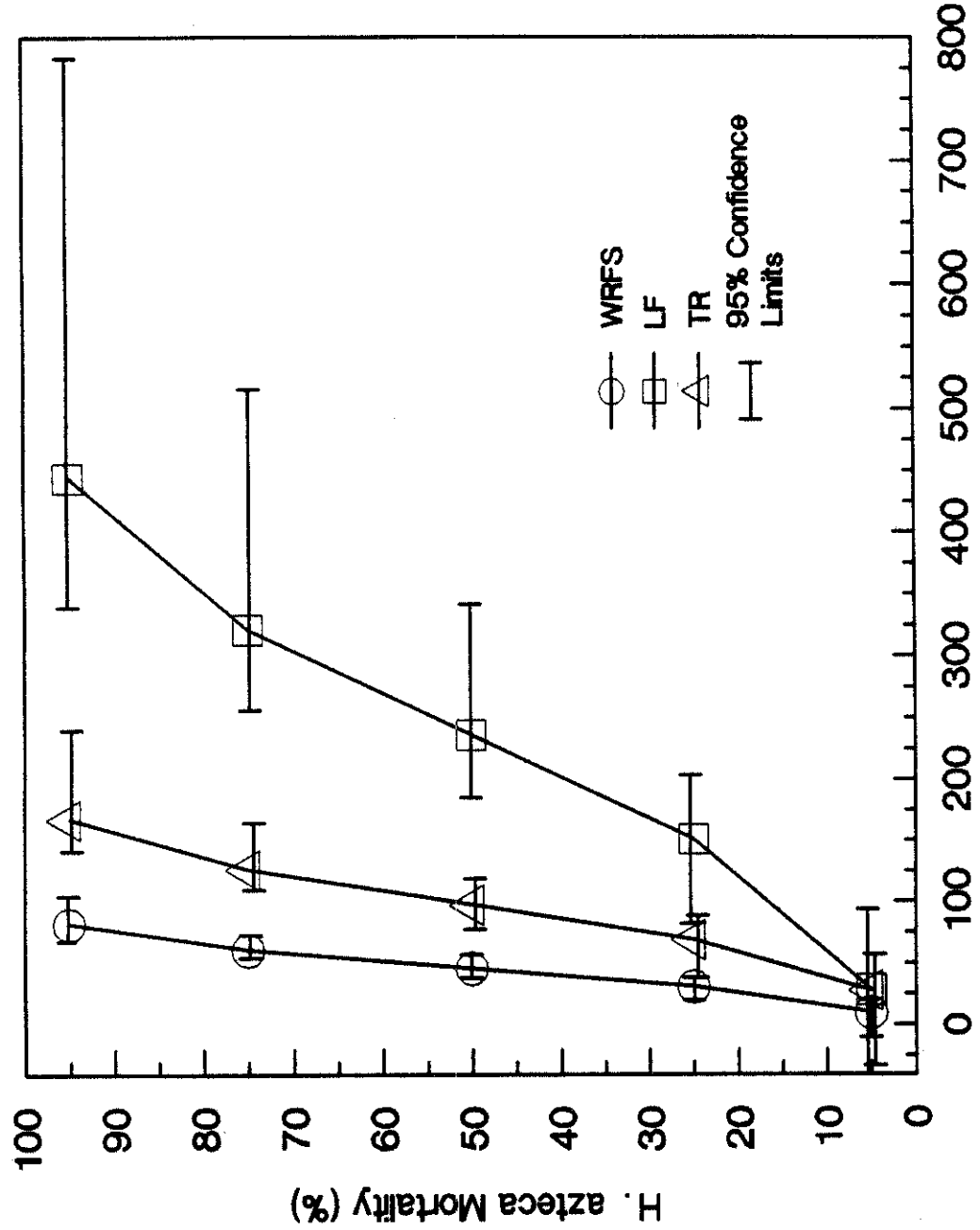


FIGURE 9. Mortality Response of Hyalella azteca Versus Fluoranthene Concentration in Pore Water.

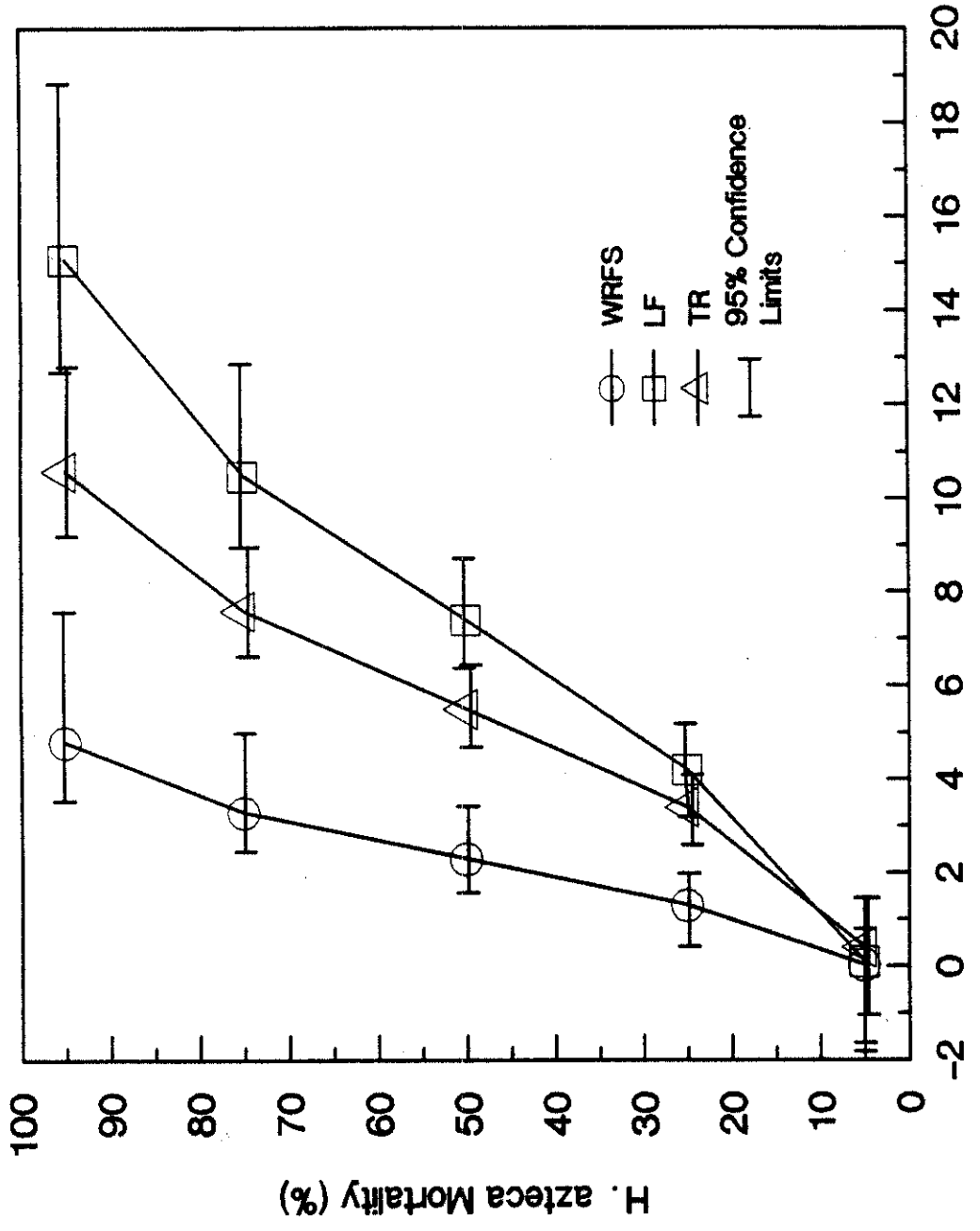


FIGURE 10. Mortality Response of Hyalella azteca Versus Fluoranthene Concentration in Sediment.

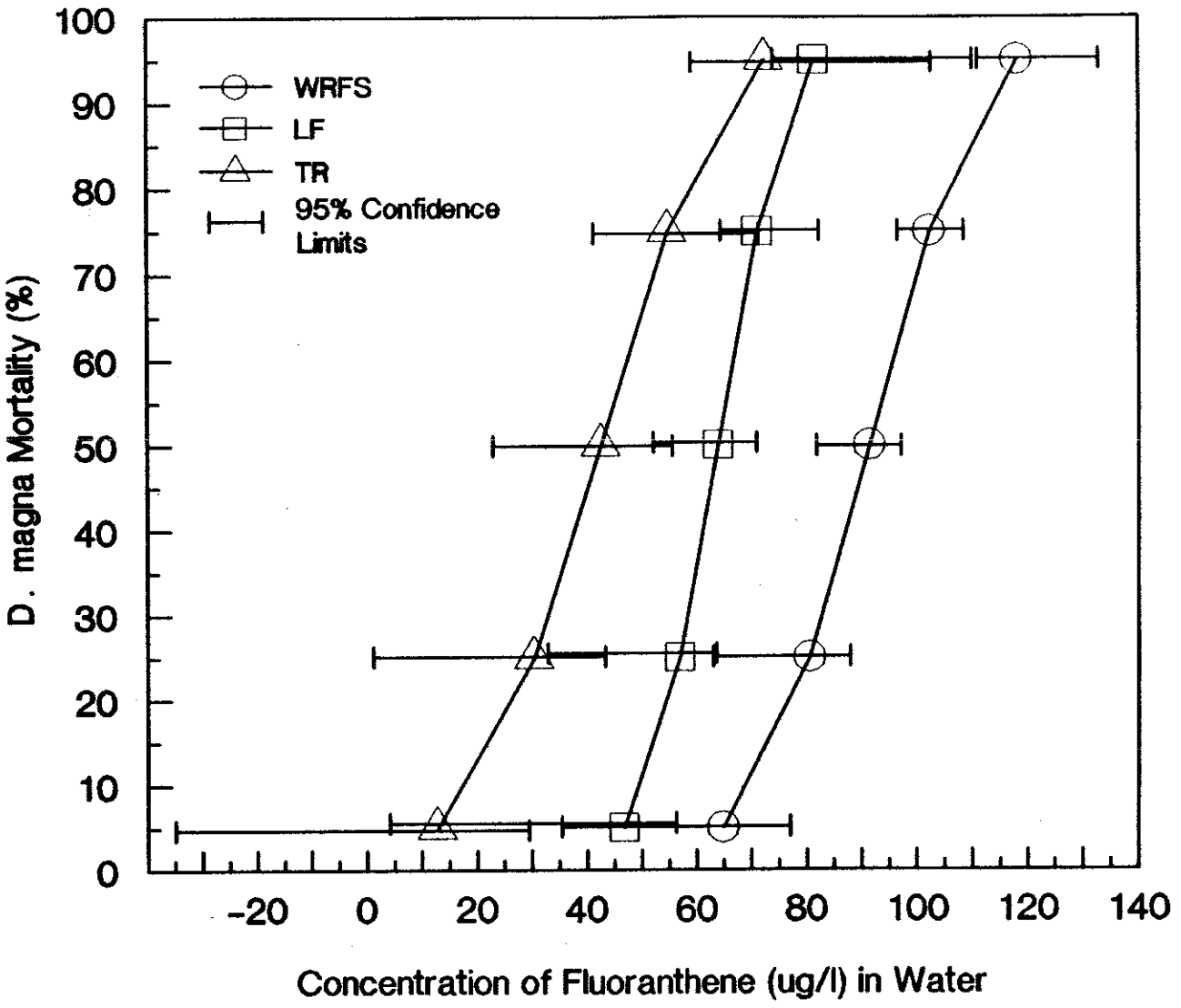


FIGURE 11. Mortality Response of Daphnia magna Versus Fluoranthene Concentration in Overlying Water.

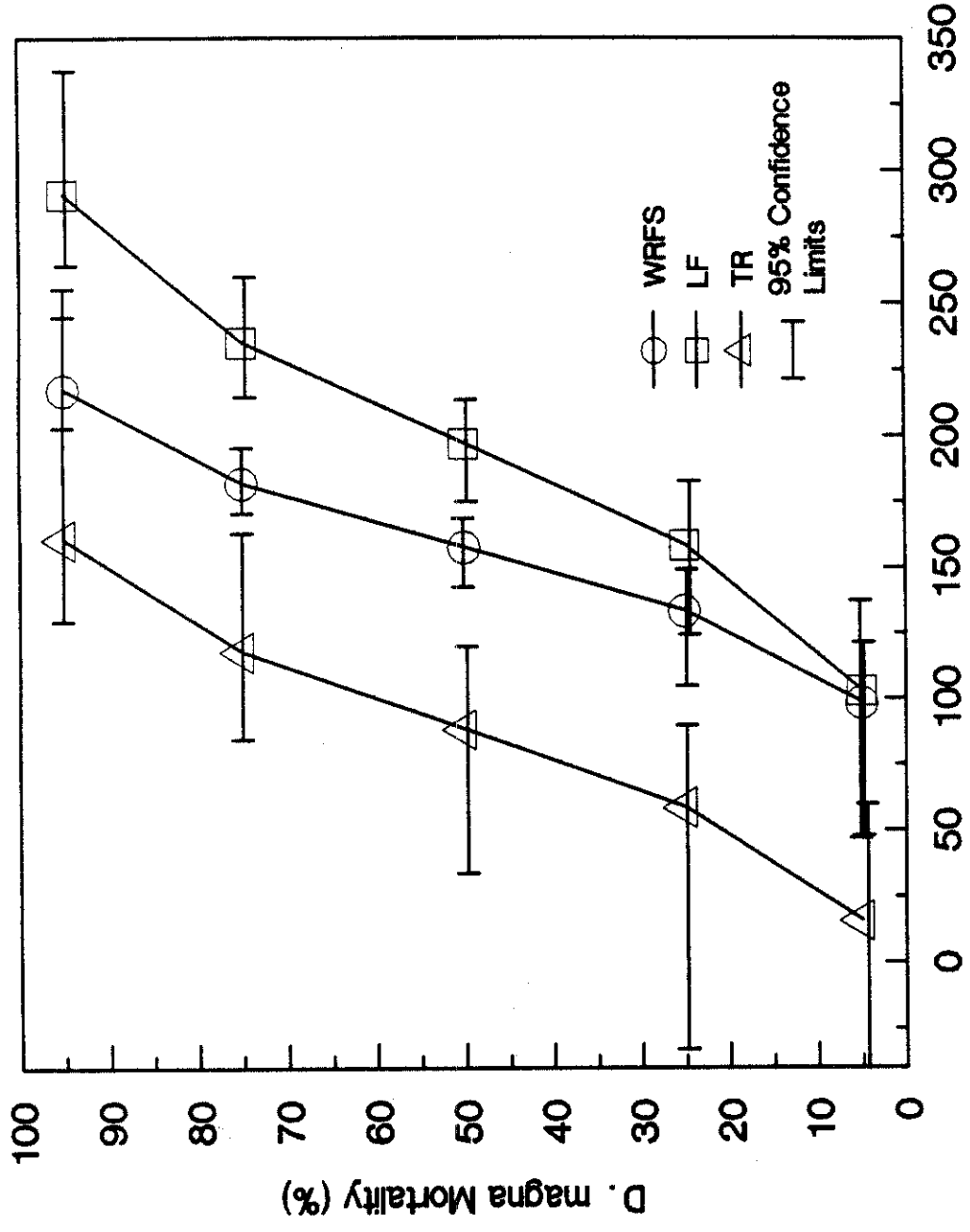


FIGURE 12. Mortality Response of Daphnia magna Versus Fluoranthene Concentration in Pore Water.

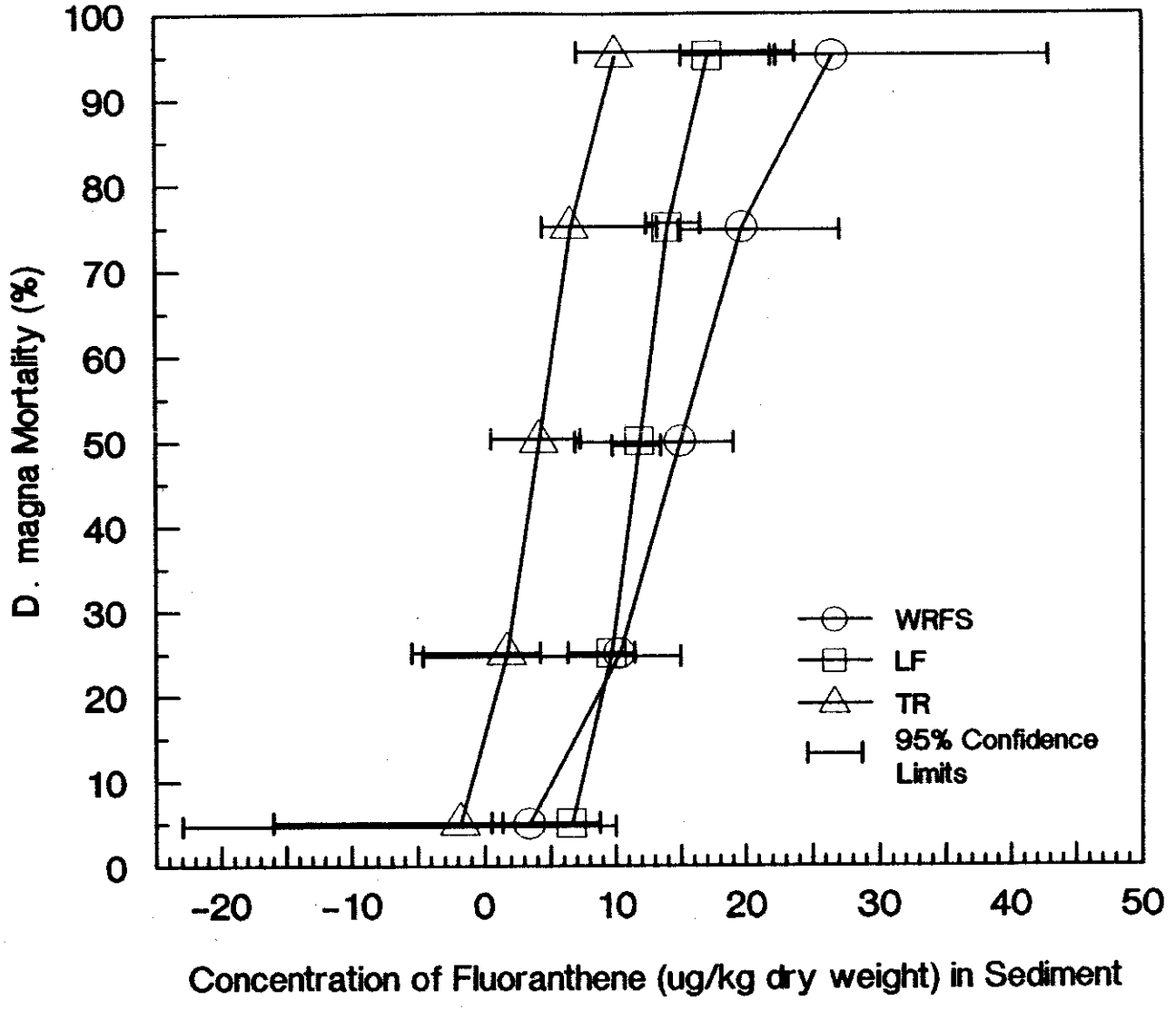


FIGURE 13. Mortality Response of Daphnia magna Versus Fluoranthene Concentration in Sediment.

CHAPTER 4

DISCUSSION

Sediment Characterization

Organic carbon (OC) values of freshwater sediments collected during this study were similar to organic carbon values from other studies reporting OC data (Table XXIV). Ritchie (1989) reported a mean OC content of 1.9% in 58 sediments collected from small reservoirs throughout the country. As previously indicated, Adams (1987) reported a mean OC content of 2.0% (N=187) from sediments throughout the country. Karickhoff (1981) reported a mean OC value of 1.36% in sediments collected throughout the U.S. Kelly and Hite (1981) reported a mean OC content of 4.6% from 63 Illinois lakes. Means et al. (1980) found an OC content of 1.38% in sediments collected from the Ohio, Missouri, Mississippi and Illinois rivers and their watersheds. However, Barko and Smart (1986) measured a higher mean OC content of 12.1% in freshwater sediments collected from around the U.S. and Canada.

Marine sediments collected during this study had a slightly lower OC content than freshwater sediments. In another study, fifteen stations sampled along the New Jersey coast had a mean OC content of 0.37% (C. Missimer, personal

TABLE XXIV. Comparison of Means of Freshwater and Marine Sediments Collected from Various Sources Sampled Throughout the United States.

Parameter	Mean	Range	N	Reference
Organic Carbon (%) Freshwater	2.0	-	187	Adams, 1987
	1.36	0.15 - 2.38	14	Karickhoff, 1981
	4.6	0.30 - 14.2	258	Kelly and Hite, 1981
	1.38	0.15 - 2.38	11	Means, et al. 1980
	12.1	0.5 - 34.0	39	Barko and Smart, 1986
	1.9	0.3 - 5.6	58	Ritchie, 1989
	1.36	0.03 - 11.8	127	This study
Organic Carbon (%) Estuarine	0.37	0.07 - 1.41	15	C. Missimer, pers. comm.
	1.14	0.02 - 6.35	26	This study
CEC (me/100g) Freshwater	13.2	1.2 - 33.0	14	Karickhoff, 1981
	28.0	6.8 - 46.2	22	Toth and Ott, 1970
	14.6	3.7 - 33.0	11	Means, et al. 1980
	21.4	5.0 - 54.0	58	Ritchie, 1989
	5.5	0.1 - 59.2	127	This Study

communication). Eighty - eight percent of the OC values obtained during this study for both freshwater and estuarine sediments combined were less than 3% OC and 93% of the OC values collected were less than 5% OC.

Cation exchange capacity (CEC) determined for sediments in this study was lower than CEC values reported elsewhere. For samples collected during this study (freshwater and estuarine combined), 98% of the CEC values were between 0.1 and 35 me/100g. Karickhoff (1981) reported values with a mean CEC of 13.2 me/100g, while Toth and Ott (1970) presented a CEC of 28.0 me/100g from 22 sediment samples collected in the Northeastern U.S. Ritchie (1989) reported a mean CEC value of 21.4 for sediments from reservoirs throughout the country. Marine sediments were also reported (Toth and Ott, 1970) with a mean of 59.7 me/100g for Chesapeake Bay and Barnagat Bay samples but these samples were not washed to remove chloride as in the present study. Samples collected by Means et al. (1980) had a mean CEC of 14.59 me/100g.

CEC values for freshwater sediments (mean = 5.5 $\pm$ S.D. = 8.3) were similar to CEC values from estuarine sites (mean = 3.6 $\pm$ S.D. = 5.8).

CEC was not observed to be positively correlated with clay content in this study (Table XIII) probably because the type of clay material is also important in determining the

CEC (Brady, 1974; Toth and Ott, 1970; Bailey and White, 1964). Clay minerals such as vermiculite and montmorillonite have CEC's ranging from 80 to 150 me/100g and surface areas ranging from 6 - 8 x 10^6 cm^2/g while clay minerals such as chlorite and kaolinite have lower CEC's (3 to 40 me/100g) and lower (7 - 40 x 10^4 cm^2/g) surface areas (Bailey and White, 1964).

Redox potentials for freshwater and estuarine sediments on average, indicated reduced sediments (Bohn, 1971) with freshwater sediments having slightly lower redox potentials (Tables VI and VII). Sediment redox potential effects on biodegradation have been noted for some synthetic organics (Gambrell et al., 1984).

Since particle surface area was observed to vary several orders of magnitude, it has logical potential as a sediment toxicant bioavailability normalization factor. Surface area of soils is reported to be positively correlated with CEC ($r = 0.985$) and percent clay ($r = 0.918$) (Mortland, 1954). However, surface area was not found to be correlated to CEC or percent clay in this study ($r = -0.136$, $N = 370$). Particle surface area depends on both size and shape of sediment particles as well as type and amount of clay present (Hillel, 1971; Brady, 1974). Clay minerals such as montmorillonite and vermiculite have surface areas on the order of 800 m^2/g and extensive internal surfaces due to

their sheetlike structure. Each particle is made up of numerous plate-like crystal units with negative charges that attract cations and water (Brady, 1974). Clay minerals such as kaolinite have only external surfaces and much lower surface areas (10 to 70 m^2/g) (Hillel, 1971; Bailey and White, 1964; Carter et al., 1986); thus, the potential for binding neutral organic chemicals is much greater in sediments with greater particle surface areas (greater CEC). Silt and sand particles have particle surface areas on the order of 0.045 m^2/g and 0.0045 m^2/g, respectively, and therefore would be expected to provide relatively few binding sites for hydrophobic compounds (Karickhoff et al., 1979).

Percent organic carbon was highly significantly correlated with CEC and highly negatively correlated with percent solids and percent sand (Table XIII). Ritchie (1989) reported that percent OC is positively significantly correlated with CEC ($r = 0.31$, $p = 0.01$, $N = 58$). Percent OC was reportedly highly correlated with CEC in other studies as well (Karickhoff, 1981; Bailey and White, 1964). The highly negative correlations between OC content and CEC with sand content (Table XIII) suggest that very little of the OC is associated with the sand fraction but rather with the silt and clay fractions. A significant positive correlation between CEC and percent clay has been noted elsewhere

(Mortland, 1954) but was not observed in this study. This is probably due to the varying types and amounts of clay minerals present in the sediments collected and because CEC largely depends on the high surface area clay minerals such as montmorillonite (Dyal and Hendricks, 1950).

Few studies have focused on effects of OC content or other sediment characteristics on bioavailability of potentially toxic materials sorbed to sediments. Staples et al. (1985) hypothesized that bioavailability of neutral organic compounds is dependent upon suspended solids concentration and is a function of organic carbon content and sorption coefficient (Kp) of the chemical. However, their data did not support this hypothesis. Adams (1984) suggested that no-effect concentrations were dependent upon OC content in sediment studies using Chironomus tentans. Bioavailability of organic compounds has been linked to solubility, sediment particle size and OC content of sediments (Schuytema, et al., 1988). The hypothesis that sediment toxicity due to a particular chemical does not vary if OC content is held constant while varying other sediment characteristics has apparently not been tested until now. These data now permit experimental examination of this and other hypotheses relevant to development of scientifically defensible SQC.

Toxicity Testing

The ten day aqueous EC50 value for 3 day old Daphnia magna exposed to fluoranthene was 102.6 ug/l. Preliminary testing using <24 h old Daphnia neonates showed a 48 h EC50 of 91.9 ug/l (95% C.I. = 77.4 - 108.3). Previous studies using D. magna exposed to fluoranthene in 48 h static tests reported a 48 h EC50 value of 325,000 ug/l (U.S. EPA, 1980) which is well in excess of the present study's observed aqueous solubility of fluoranthene (150 ug/l).

The organic carbon normalization approach implies that the bioavailable fraction of a neutral organic compound (fluoranthene) in sediment is governed solely or largely by the total organic carbon content of that sediment. Theoretically, if the organic carbon content increases, then the toxicity of fluoranthene should decrease. Conversely, if organic carbon content of sediment is held constant (as in this study), then observed toxicity should remain constant. The objective of this study was to select three sediments with similar organic carbon contents while varying other sediment characteristics (Table III). However, by holding organic carbon content constant, it was difficult to vary other sediment characteristics by an order of magnitude while at the same time having a sediment that exhibited no toxicity to the organisms tested. Since all sediment characteristics studied were essentially the same, the

variance observed between sediments for each organism is due to factors other than the sediment characteristics examined in this study.

As illustrated in Figures 5 - 13, significant differences occurred between sediments for all three organisms tested for overlying water, pore water, and sediment fluoranthene concentrations. These data indicate that bioavailability of fluoranthene to D. magna, H. azteca and C. tentans is mediated by factors other than sediment organic carbon content alone. The data also illustrate that the other sediment characteristics studied did not vary sufficiently to account for the significant differences observed between the toxicity mediating properties of the sediments.

One of the assumptions of the Equilibrium Partitioning approach to SQC development is that water column organisms have the same sensitivities as infaunal benthic organisms (Chapman, 1989). The data from this study of fluoranthene based on 10 day EC50s (Table XIV) indicate that benthic species such as C. tentans and bottom dwelling species such as H. azteca are as sensitive or more sensitive than water column organisms such as D. magna.

The findings of this study differ from other studies on the effects of organic carbon on bioavailability of neutral organic compounds to aquatic organisms. Adams et al. (1985)

found that C. tentans was affected biologically only when the pesticide, Kepone (K_p = 120), sediment pore water concentrations exceeded the chronic water effect level for Kepone. Adams et al. (1985), and Ziegenfuss et al. (1986) observed that overlying water concentrations were very similar to pore water concentrations in static tests using sediments with varying organic carbon contents. Ziegenfuss et. al. (1986) also noted that LC50 values based on pore water concentrations were the same as those based on overlying water concentrations. That study supported the organic carbon normalization theory for neutral organic compounds sorbed to sediments.

Nebeker et al. (1989) exposed H. azteca to DDT and endrin in 10 day whole sediment tests. The authors observed that DDT ($K_p = 1.2 \times 10^5$) was more tightly bound to high organic carbon content sediment (10.5%) than low organic carbon sediment (3.0%) and was not bioavailable to Hyalella. These DDT data supported the organic carbon normalization approach. However, endrin ($K_p = 6.9 \times 10^3$) toxicity was observed not to be affected by sediment organic carbon content, thus contradicting the organic carbon normalization approach to SQC development.

Data from this study do not support the organic carbon normalization approach. Fluoranthene sediment pore water concentrations were not similar to overlying water

concentrations in any of the tests conducted, even though organic carbon content was held constant. EC50 values based on sediment pore water concentrations were not similar to EC50 values based on overlying water concentrations. One of the major assumptions of the Equilibrium Partitioning approach is that sediment pore water concentrations can be calculated directly from the amount of chemical sorbed to sediments and percent OC (Chapman, 1989). Figure 14 demonstrates that fluoranthene sediment pore water concentrations vary by up to a factor of four (taking fluoranthene sediment concentrations into account), illustrating that fluoranthene sediment pore water concentrations cannot be accurately calculated from the amount of fluoranthene sorbed to sediments.

Based on the above discussion, the mediating effects of organic carbon were not dependent upon the chemicals' partition coefficient (K_p). The partition coefficients for Kepone (K_p = 120) and DDT (K_p = 1.2 x 10^5) that supported the EP approach differed by three orders of magnitude. Partition coefficients of fluoranthene and endrin that do not support the EP approach were very similar (K_p = 1 x 10^3) and were between the coefficient values for Kepone and DDT. These data suggest that organic chemical partition coefficients do not account for the observed differences in toxicity of organic compounds sorbed to sediments.

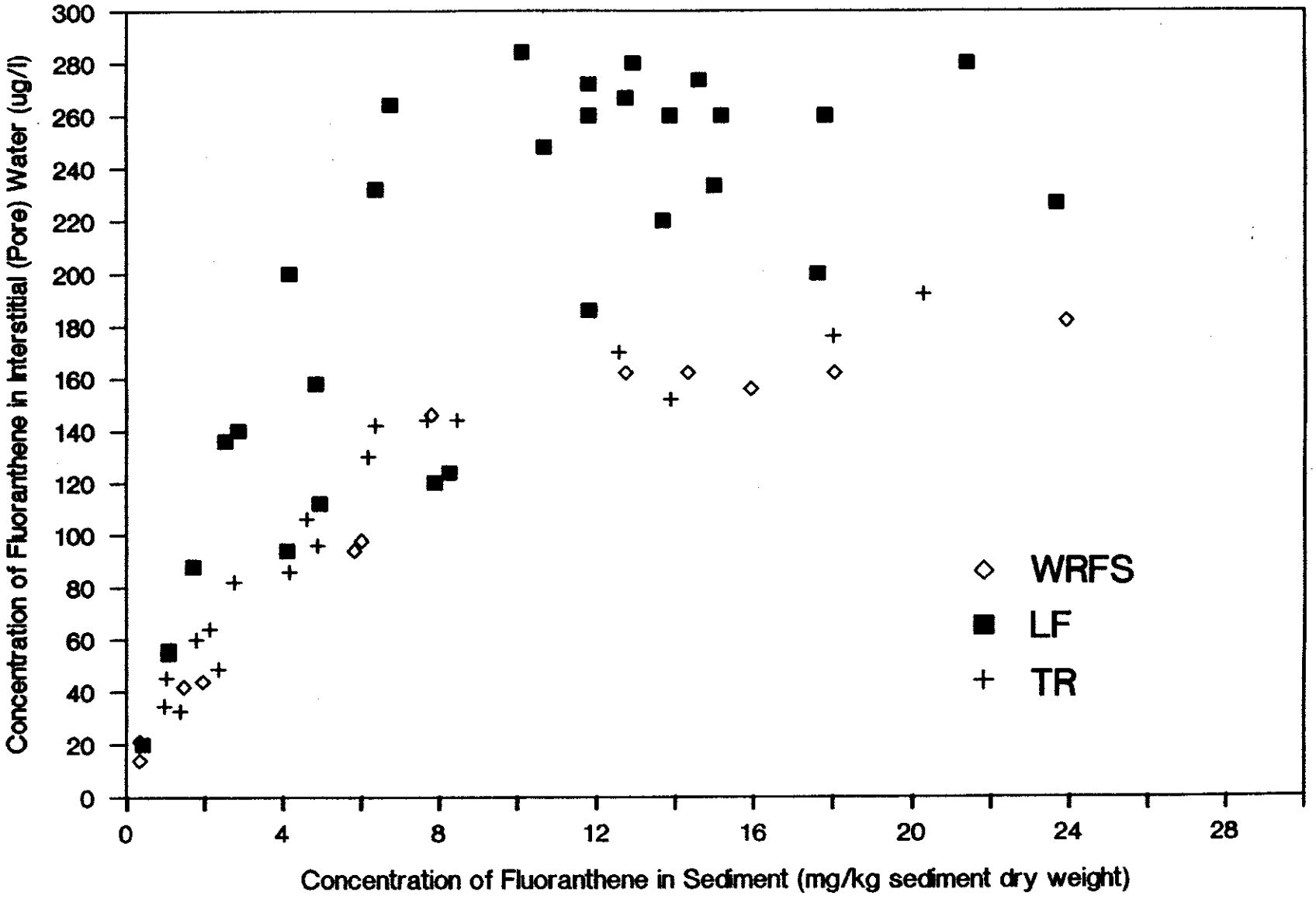


FIGURE 14. Plot of Fluoranthene Pore Water Concentrations Versus Fluoranthene Sediment Concentrations for All Three Sediments Used During This Study.

One subject that has not been addressed is whether or not chemical sorption and bioavailability are similarly affected by differing forms of organic carbon. As noted in Table VI, coal was found in two sediment samples. Does coal affect chemical sorption and bioavailability the same as dissolved and particulate organic carbon? (e.g. - are all forms of organic carbon created equal?). Answers to these questions must be addressed in order to develop defensible SQC.

Conclusions

1) Percent organic carbon, cation exchange capacity and particle surface area were the only sediment characteristics studied that varied sufficiently to encompass the range in observed sediment toxicity.

2) Sediment characteristics studied for both freshwater and marine sediments (organic carbon, cation exchange capacity, particle size distribution, particle surface area, redox potential, and percent sediment solids) were found to be statistically significantly different within and among most physiographic provinces.

3) Freshwater sediment characteristics did not differ significantly from estuarine (marine) sediment characteristics.

4) These data suggest that there is no significant correlation between sediment toxicity and the bulk chemistry of sediments (organic carbon, cation exchange capacity, particle size distribution, particle surface area).

5) Total organic carbon content of sediments varies sufficiently (two orders of magnitude) to be used as a potential normalization factor for the bioavailability of potentially toxic materials in sediments.

6) Sediments with similar organic carbon contents do not necessarily possess similar toxicity mediating properties for neutral organic compounds (e.g. fluoranthene).

APPENDIX A

SEDIMENT COLLECTION SUMMARY SHEET

Sediment Collection Summary Sheet

1) Sediment particle size must be <2 mm diameter.

2) Sediments should be kept on ice or refrigerated at 4°C if possible - Please Don't Freeze Sediment!

Province:

Site Name:

Town/County/State:

River/Lake/Bay Name:

Highway #:

Date Collected:

Collected By:

Sediment Distingiushing Characteristics (if any):

If you have any questions, please call:

Burton Suedel
(817) 565-3599
Thank You!

APPENDIX B

SEDIMENT ANALYSIS PROCEDURES

SEDIMENT DISTRIBUTION FOR EACH PARAMETER

1. Homogenize sediment sample thoroughly with spatula or power drill and impeller.

2. Sieve sediment through a #10 U.S.A. Standard Testing Sieve (2.00 mm opening) to remove particles larger than coarse sand.

3. Homogenize sediment again to ensure complete mixing.

4. Need approximately 5g wet (X3 reps) in aluminum pans for percent solids.

5. Need 5.0g wet (X3 reps/sediment) for CEC.

6. Need wet sediment (100 ml) in sample container for redox potential.

7. Need approximately 2g wet (X3 reps) for percent organic carbon determination (does not need to be weighed).

8. Dry sediment (35g) equivalent* for particle size in a 200 ml Beaker.

*Weigh out wet sediment and divide by percent water (decimal fraction) to obtain dry weight equivalent of sample.

PERCENT SOLIDS (PERCENT WATER)
(after Black, 1986)

1. Homogenize sediment sample.

2. Weigh out approximately 5g wet sediment sample on a tared aluminum foil dish to the nearest 1.0 mg (3 reps per site). Do not touch aluminum pan with fingers - use tongs.

3. Dry samples approximately 24h in a drying oven at 103-105^{0}C.

4. Remove samples from drying oven and let cool in a desiccator. Weigh the samples on the same balance used to obtain the wet weight.

5. Repeat the drying process until a constant weight is obtained.

6. The total solids of the sediment samples are calculated by dividing the weight of the dried residue by the initial weight of the sample:

$$\% \text{ Solids} = \frac{\text{sed dry wt (after)}}{\text{sed wet wt (before)}} \times 100$$

$$\% \text{ Water} = 100 - \% \text{ Solids}$$

CATION EXCHANGE CAPACITY
(after Plumb, 1981)

The cation exchange capacity (CEC) of a sediment is a measure of the reversibly bound cations in the sample, or, a measure of those cations held on the surface within the crystalline matrix of some minerals. These cations may potentially be released to the water column under appropriate conditions.

The procedure consists of equilibrating a sediment sample with a highly soluble salt solution. The theory behind the procedure is that the high concentration of a soluble cation will replace the sorbed or bound cations associated with the sediment. The replaced cations can then be determined individually in the leachate or the sediment sample can be washed and reequilibrated with a second soluble salt. The second leachate is then analyzed for total cation exchange capacity. The standard leachate that is most often used is 1 N ammonium acetate. The principle advantages of this approach are the pH buffering capacity of ammonium acetate solutions and the relative ease of the ammonia determination. However, ammonium acetate may yield low results with (a) samples containing 1:1 type clay minerals such as kaolin or halloysite, or (b) highly calcareous sediments due to the dissolution of calcium carbonate.

The exchange capacity of a sample is influenced by the clay content of the sample, the type of clay, the organic matter content, the pH of the displacing solution, the nature and concentration of the displacing cation, and the sediment-to-solution ratio. Since many of these factors are operationally defined, the CEC of the sample should be considered operationally defined. Most techniques will only vary the magnitude of the CEC of a sample and not the relative order of a number of samples. Therefore, care should be taken to standardize as many variables as possible (ammonia concentration, pH, solid-liquid ratio, and time of contact) to ensure uniformity and comparability of results.

Sample Handling and Storage

Samples may be collected with any convenient collection device and stored in either glass or plastic containers. Field moist samples should be used for the CEC determination as the process of drying has been shown to alter the CEC. At this time, the storage time limits are not known. Since sample oxidation may indirectly affect CEC, it is recommended that samples be processed as soon as possible.

Reagents Needed:

1N NH_4OAc: Dissolve 77g of ammonium acetate in Milli Q (MQ) or deionized water (DI) and dilute to 1 liter.

80% ethanol: Mix 80 ml ethanol in 100 ml volumetric flask and dilute to 100 ml with MQ water.

10% NaCl: Dissolve 100g of NaCl in MQ water; dilute to 1 liter.

* Note: If marine or brackish sediments are used, wash the sediments with DI water until there is no trace of chloride.

Procedure:

1. Weigh out a 5.00g sample of homogenized sediment in a 40 ml polypropylene centrifuge tube. Do three replicates per site.
2. Add 33 ml of 1N NH_4OAc solution.
3. Shake sample(s) on a vortex mixer for 15 seconds and let the suspension stand for 30 minutes.
4. Vortex mix the suspension again for 15 seconds.
5. Centrifuge for 5 minutes at 1600 rpm (300 xg).
6. Decant the NH_4OAc solution.
7. Repeat 2X (#3-#7).
8. Add 33 ml 80% ethanol to the sediment residue in the centrifuge tube.
9. Vortex mix the tubes.
10. Centrifuge for 5 minutes at 1600 rpm.
11. Decant the ethanol layer and discard (note yellow color of ETOH).
12. Repeat 2X (#9-#11).

13. Add 33 ml of 10% NaCl solution to the washed sediment residue. Vortex mix.

14. Centrifuge the sample for 5 minutes at 1600 rpm.

15. Decant the liquid phase into a 125 ml erlenmeyer flask.

16. Repeat 2X (#13-#15).

18. Dilute the erlenmeyer flask to volume (100 ml) with NaCl.

19. Analyze the sediment leachate for ammonia.

$$\text{CEC (me/100g)} = \frac{(X \text{ mg/L})\ (0.1)\ (100)}{(18 \text{ mg/meq})\ (g)\ (\%S)}$$

Where:

X = ammonia concentration in NaCl leachate, mg/L

0.1 = volume of NaCl leachate, liters

18 = millequivalent weight of ammonium ion, mg/me

g = weight of wet sediment sample, grams

%S = percent solids (% dry weight) in sediment sample as a decimal fraction

AMMONIA ANALYSIS
FOR CATION EXCHANGE CAPACITY DETERMINATION
(Standard Methods 15th Ed. pp. 362-363)

The ammonia-selective electrode uses a hydrophobic gas-permeable membrane to separate the sample from an internal solution. By increasing the pH of the sample to above 11 with a strong base, dissolved ammonia is converted to NH_3 (aq.). This diffuses through the membrane altering the pH of the internal solution, which is sensed by a pH electrode.

STORAGE AND MAINTENANCE

The ammonia electrode (Orion Model 95-12) is stored in 0.1M stock standard or the 1000 ppm stock solution. Replace the storage solution when it becomes colored. The probe membrane must be replaced periodically. The probe manual gives a range of one week to several months, depending on use. Membranes are expensive and a replacement supply requires 3-4 weeks to be filled; so use them judiciously. To replace the membrane, unscrew the top cap and remove the inner electrode body, pour out the inner filling solution, remove the bottom cap and the old membrane. Using forceps to hold the edge of the membrane, loosely stretch the membrane across the bottom of the outer body. Place the cap over the ends and screw it on finger-tight. The membrane should be wrinkle-free. Using the syringe, put 2.5 ml of the internal filling solution (Orion 95-12-02) into the outer body. Place inner body into the outer body and screw on the upper cap. Shake the fully assembled electrode as if it were a clinical thermometer. Use an electrode stand that holds the probe at a 20° angle to prevent air entrapment. Prior to running samples, warm the ammonia ISA and standard to the same temperature as the samples to be tested.

Ammonia Stock Standard:

3.819 g anhydrous, reagent-grade NH_4Cl (oven dry at 100 + 5°C for 24h) brought to 1000 ml with MQ water. This yields a 1000 ppm solution. 1 ml = 1 mg NH_3-N = 1.22 mg NH_3

Ammonia ISA (ionic strength adjuster)

400 g NaOH in 800 ml ammonia free water (MQ). Cool and dilute to 1000 ml. This yields 10M NaOH. This ISA does not require refrigeration.

SLOPE CHECK - AMMONIA

1. Rinse all glassware with MQ water.

2. Warm samples to 20°C by placing them in a warm water bath. Monitor the temperature by placing a thermometer in the mouth of one of the sample bottles.

3. Rinse the ammonia probe with MQ water, gently wipe with a Kimwipe and place in a beaker with pH 4 buffer.

4. Plug probe into meter (Corning Model 135 pH/ion meter).

5. Make sure the meter is clear by pressing SLOPE. If a number appears press RESET (RESET clears all stored data).

6. Put mode on Mv by pressing MODE button until the red light is next to Mv.

7. Press 0, CAL 1.

8. In a 150 ml beaker rinsed with MQ water add 100 ml of MQ water and 1.0 ml 1000 ppm ammonia stock standard.

9. Place the beaker on a stir plate, rinse the stir bar with MQ water and place it in the beaker. Stir so as not to have a vortex.

10. Rinse the probe with MQ water, gently wipe and place in the beaker.

11. Add 1.0 ml ISA (10M NaOH) to the beaker and press READ.

12. Press CAL 1 and then CLEAR when the digital readout remains constant for 4 flashes of the red light.

13. Without removing the probe, add an additional 10 ml of the ammonia stock and press READ.

14. Wait for the numbers to stabilize. The digital readout should display -57.00 $\pm$ 3.

If the readout deviates considerably (less than -60 or greater than -50), soak the probe in pH 4 buffer for 10 minutes, redo the slope check, and see the trouble shooters section of the ammonia probe users manual.

CALIBRATION

1. Press CLEAR.

2. Rinse the ammonia probe with MQ water, gently wipe and place in a small beaker with pH 4 buffer.

3. Rinse three volumetric flasks (100 ml) with MQ water and then fill each with approximately 85 ml of MQ water.

4. Add 1.0 ml of the 1000 ppm ammonia stock standard to the volumetric flask marked 10 ppm. Bring to volume with MQ water, cover with parafilm and invert several times.

5. Take out 10 ml of the 10 ppm dilution and add it to the volumeteric flask marked 1.0 ppm. Bring to volume with MQ water, cover and invert several times.

6. Take out 10 ml of the 1.0 ppm dilution and add it to the volumetric flask marked 0.1 ppm. Bring to volume with MQ water, cover and invert several times.

7. Rinse two 150 ml beakers with MQ water. Pour the entire contents of the 1.0 ppm dilution into one beaker and the contents of the 0.1 ppm dilution into the other beaker.

   Keep separate.

8. Change the mode of the meter to ACTIVITY by pressing MODE until the red light is next to ACTIVITY.

9. Press 1.0 CAL 1, 0.1 CAL 2, -60.00 SLOPE.

10. Check by pressing CAL 1, CAL 2, SLOPE.

11. Place the beaker with the 1.0 ppm dilution on the stir plate. Rinse the stir bar and place in the beaker.

12. Rinse probe, gently wipe and place in the beaker.

13. Add 1.0 ml ISA to the beaker and press READ.

14. When the number stabilizes for 4 flashes of the red light, press CAL 1.

15. Press CLEAR, remove the probe, rinse and gently wipe and place it in the pH 4 buffer. Place the beaker with the 0.1 ppm dilution on the stir plate. Place a rinsed stir bar in the beaker.

16. Remove probe from the buffer, rinse and wipe and place in the beaker.

17. Add 1.0 ml ISA to the beaker and press READ. When the number stabilizes for 4 flashes press CAL 2.

18. Press CLEAR, then SLOPE. The number should read -57.00 $\pm$ 2. If the reading deviates considerably (less than -60 or greater than -50), check dilutions, check the trouble shooters section of the users manual, recalibrate.

Note: Always calibrate with the highest dilution first.

READING SAMPLES

1. Warm samples to 20°C by placing bottles in a warm water bath. Monitor the temperature by inserting a thermometer into the mouth of one of the sample bottles.

2. Rinse a 150 ml beaker with MQ water. Add 100 ml of sample.

3. Place the beaker on the stir plate. Rinse the stir bar and place in the beaker. Stir so as not to have a vortex. Place probe in beaker.

4. Add 1.0 ml ISA. Press READ.

5. Record reading after the numbers stabilize (4 flashes of the red light).

6. Press CLEAR.

7. Remove the probe, rinse, wipe and place in the pH 4 buffer.

8. Repeat steps 2-6 for each sample.

9. When samples are completed, rinse and gently wipe the probe. Place it in 0.1M stock standard or 1000 ppm stock. Turn off the stir plate and unplug the probe.

   Rinse all glassware with MQ water.

Note: If the sample reading is greater than the calibration range of the meter (i.e. calibration 0.1-1.0 and reading of 5.6) dilute the sample, take a reading and note the dilution

factor. The meter can also be calibrated to the anticipated range of the samples. Recalibration may be necessary.

Note: If reading is below 0.01 then it is below the detection limit.

STANDARD ADDITION SPIKE

Some of the samples in the sample run should be "spiked" to find the range of detection. After the sample has stabilized, the standard addition spike is placed in the sample and read. The standard addition should not exceed the detection limit. An example of a standard addition spike follows:

In a 10 to 1 calibration, a 0.5 ppm spike may be used (1 ml of a 10 ppm standard dilution). This addition should increase the sample reading 0.1 ppm.

PARTICLE SIZE

SEDIMENT PARTICLE COMPOSITION ANALYSIS - HYDROMETER METHOD (after Black, 1986)

Particle size distribution is a cumulative frequency distribution or a frequency distribution of relative amounts of particles in a sample within a specified size range. The size of a discrete particle is usually characterized as a linear dimension and designated as a diameter. It should be recognized that the use of sieves and settling tubes will result in a separation based on particle shape as well as particle size.

The size distribution of sediments can be of importance because it can affect the distribution of chemicals in the aquatic environment. Specifically, sediments can remove chemical contaminants from water by the process of sorption. Further, since sorption is a surface phenomena, the smaller particle sizes generally have a higher concentration of these chemical contaminants on a weight/weight basis.

There is a certain amount of arbitrariness associated with particle size analysis. One method relies on the treatment of the sample with hydrogen peroxide to destroy organic matter that may be causing the sediment particles to aggregate. While this approach will define the true particle size distribution of the sample, the results will not be representative of the surface area potentially available for sorption or exchange reactions. On the other hand, sizing of sediments without peroxide treatment would yield results more representative of the exposed surface area but the apparent particle size distribution may be affected by the method of sample handling prior to sizing. The latter procedure will be used in this study.

Sample Handling and Storage

Samples scheduled for particle size analysis may be stored in either plastic or glass containers. The samples should be chilled at 4-5^{0}C but never frozen prior to analysis. If samples cannot be analyzed within a few hours, Lugols solution should be added as a preservative to minimize the effects of bacterial growth (not used in this study).

The required amount of sediment will range from approximately 35 to 45g (dry wt), depending on the size distribution. Should the sample contain a large percentage of coarse sand and gravel, a larger sample size should be

used to ensure that the smaller size classes are being representatively sampled.

It is recommended that particle size samples not be frozen or dried prior to analysis. The basis of this recommendation is that the freezing-thawing cycle or sample drying may cause an irreversible change in the particle size distribution due to oxidation and/or agglomeration.

Particle size distribution is as follows:

Grade	Particle Diameter (mm)
Course sand	0.2 - 2.0
Fine sand	0.02 - 0.2
Silt	0.002 - 0.02
Clay	< 0.002

Equipment:

1. Balance - sensitive to 0.01g
2. Hydrometer
3. Several 1 liter graduated cylinders - one blank and 3 cylinders/sediment site
4. Thermometer
5. 16,000 rpm blender
6. Timer
7. 250 ml beakers
8. Squirt bottle
9. Sediment plunger
10. n-amyl alcohol

Reagents:

Sodium hexametaphosphate $(Na_3PO)_6$ (HMP) 5% molal solution

(w/vol) - 50g HMP weighed out and diluted to 1 liter with deionized (DI) water.

Procedure:

1. Wet sediment sample is sieved to 2 mm particle size with a #10 sieve.

2. Place > 45g of wet sediment in a tared 250 ml beaker (X3 reps). Divide target dry weight (35g) by solids fraction to obtain wet weight needed. Record sediment weights. Add 100 ml of HMP solution to the samples. Mix thoroughly with a spatula and cover. Allow sediment to soak in the HMP solution between 15 to 24 hours.

3. Sediment and HMP are then rinsed into a blender (Hamilton Beach, Scovill Model 936.1) with DI water, rinsing beaker with squirt bottle. Fill blender cup to approximately 1-2 inches from the top with DI water. Blend for 2 minutes on "high" speed.

4. Wash contents of blender cup into a 1 liter graduated cylinder with DI squirt bottle. Fill cylinder to the 1 liter mark with DI water.

5. Prepare blank with 100 ml HMP solution and 900 ml DI water.

6. Do not use plunger on blank. Read hydrometer on blank first. Only one reading is needed on the blank at the beginning of the run.

7. Agitate sediment column with the plunger for 20 complete, even strokes.

8. Immediately after plunging, start timer.

9. If sediment mixture is frothy, add 2-3 drops of n-amyl alcohol.

10. Gently place hydrometer into cylinder and prepare to take reading.

11. Take readings at 60 sec, 3 min, 15 min, 8 hrs, 24 hrs.

12. Record temperatures at each time period when you can work them in.

13. Upon removing the hydrometer from each graduated cylinder, rinse with DI water before placing in next cylinder.

14. Subtract blank reading from each sample.

15. At the end of the 24 h reading time, sediment suspension must be sieved for sands. (Sands settle out too quickly to be measured in this analysis).

16. Pour entire contents of graduated cylinder through a #230 sieve (63 um) to separate the sand fraction from the silt and clay fractions. Rinse sand well with DI water to remove HMP and other particles. Use squirt bottle to transfer sand from the sieve into a 250 ml beaker.

17. Dry sand fraction in a drying oven at less than $100^{0}C$ overnight so as not to boil water and lose sample.

18. Weigh dry sand fraction.

19. Enter numbers in the computer program as given below.

    Target dry sediment weight (approximately 35g) divided by decimal fraction of percent sediment = amount of wet sediment to weigh out

    Wet sediment weight X decimal fraction of percent H_2O = equivalent dry sediment weight

    Dry sand weight divided by equivalent dry sediment weight = percent Sand

SAS INPUT STATEMENTS TO CALCULATE PARTICLE SIZE DISTRIBUTION

```
data partsize;
  *input sitename $ sedwt sandwt hyd1 hyd3 hyd15 hyd480
   hyd1440 temp1 temp3 temp15 temp480 temp1440 blank;

sand = (sandwt / sedwt) x 100;
clay = 0.0;
sedwtrem = (sedwt - sandwt) x 100;

hyd1 = hyd1 - blank;
hyd3 = hyd3 - blank;
hyd15 = hyd15 - blank;
hyd480 = hyd480 - blank;
hyd1440 = hyd1440 - blank;
if hyd480 > hyd1440 then
   do;

     P1 = (hyd480 / sedwtrem) x 100;
     P2 = (hyd1440 / sedwtrem) x 100;
     S1 = (49.664 - (0.29 x (hyd480 + 5))) x (1.3817 -
          (0.13 x temp480));
     S2 = (49.664 - (0.29 x (hyd1440 + 5))) x (1.3817 -
          (0.13 x temp1440));
     A = (S1 - 2) / (S1 - S2);
     B = (P1 - P2);
     C = A x B;
     clay = (P1 - C);
   end;

   silt = 100 - (sand + clay);
   keep sitename sand silt clay;
```

*INPUT VARIABLE IDENTIFICATION

sitename = name of sediment site
sedwt = total dry weight of sediment sample
sandwt = dry weight of sand fraction
hyd1 = hydrometer reading at 1 minute
hyd3 = hydrometer reading at 3 minutes
hyd15 = hydrometer reading at 15 minutes
hyd480 = hydrometer reading at 8 hours
hyd1440 = hyrdometer reading at 24 hours
temp1 etc. = temperature readings at the designated times
blank = hydrometer reading of blank (900 ml DI water + 100 ml HMP)

PARTICLE SURFACE AREA
(after Millar et al. 1965)

Particle surface area can be calculated directly from percent sand, silt and clay. Surface area (per gram dry weight) of a given sediment is determined by using the decimal fraction of each particle size category in a sample (sand fraction, silt fraction, clay fraction) and the average surface area (45, 454, 8,000,000 cm^2/g for sand, silt, and clay, respectively) of each fraction to estimate the surface area for each sample. Clay particles are assumed to have platy shapes and the sand and silt fractions are assumed to have spherical shapes.

SURFACE AREA (cm^2/g) = SAND FRACTION(45 cm^2/g) +

SILT FRACTION(454 cm^2/g) +

CLAY FRACTION(8,000,000 cm^2/g)

Therefore, estimated particle surface area using the above equation can range from 45 cm^2/g for a sediment containing 100% sand to 8,000,000 cm^2/g for a sediment consisting of 100% clay.

PERCENT ORGANIC CARBON
(after Dohrmann, 1985)

Carbon may exist in sediment and water samples as either inorganic or organic compounds. Inorganic carbon is present as carbonates, bicarbonates, and possibly free carbon dioxide. Specific types of compounds that are considered to be included in the organic carbon fraction are nonvolatile organic compounds (sugars), volatile organic compounds (mercaptans), partially volatile compounds (oils), and particulate carbonaceous materials (cellulose).

The basis of the method is the catalytic or chemical oxidation of carbon in carbon-containing compounds to carbon dioxide followed by the quantification of the carbon dioxide produced. Alternately, the carbon may be reduced to methane and appropriately quantified. It follows, then, that the distinction between inorganic carbon and organic carbon is the method of sample pretreatment. There are presently two procedures for determining this separation. One method is based on sample treatment with a strong acid. This method is employed in this procedure. Analysis of an untreated sample is a measure of total carbon while analysis of the acid-treated fraction is a measure of organic carbon. Inorganic carbon is calculated by subtraction. The second method of separation is based on differential thermal combustion with organic compounds being converted to carbon dioxide at 500 ^{0}C to 650 ^{0}C and inorganic carbon being converted to carbon dioxide at 950 ^{0}C to 1300 ^{0}C.

Sample Handling and Storage

Samples should be processed as soon as possible (within 24 h if possible) to minimize change due to chemical or biological oxidation. Atmospheric uptake of carbon dioxide is less critical since it would be evolved when the sample is acidified prior to analysis. Sediment samples for organic carbon analysis may be stored in either plastic or glass containers. Air drying of sediments may lead to low TOC results due to oxidation or volatilization. Therefore, moist storage would be the preferred method of storage.

Sediment Procedure

This is a mini-manual for the Dohrmann DC-80 carbon analyzer (for solids). Excerpt from: Dohrmann DC-80 Total Organic Carbon Systems Manual.

Principle of operation:

A sample placed in a platinum boat is combusted and oxidized at approximately 800 ^{0}C in an oxygen atmosphere. A constant flow of oxygen carries the resultant CO_2 to the non-dispersive infrared detector (NDIR). The NDIR produces an electrical output (peak) which is intergrated and scaled by the number processor and then displayed and printed in mg/kg (ppm) carbon.

I. Sample preparation and start-up:

If TOC is to be measured, the sample must first be acidified to remove inorganic carbon. Place approximately 2-3 grams of moist well-mixed sediment in an aluminum dish. Add 1 N H_3PO_4 with mixing until pH 2 or less is reached. (Effervescence upon addition of acid indicates inorganic carbon is being liberated as CO_2). Allow to dry overnight at 40^{0}C or under a hood. Next day, make a slurry with a small portion of the dried sediment and MQ water. Check the pH. If sample is at pH 2 or less continue with procedure. If pH is greater than 2 add more acid until a pH 2 or less is reached and allow to dry overnight again. Thoroughly grind and mix dried sediment (including salt crystals that may have formed on top) using a mortar and pestle.

Prepare reagents:

20g potassium persulfate ($K_2S_2O_8$) + 5 ml 1 N H_3PO_4. Bring up to 1 L with MQ water. Use this reagent to fill the reaction vessel.

174 ml of 85% H_3PO_4 per liter of MQ water to make 1N H_3PO_4.

Check "U" trap tube - add water if necessary.

Prepare standard:

To make 2000 ppm standard: Add 0.425g potassium hydrogen phthalate (potassium biphthalate)($C_8H_5O_4K$) and 1 ml 1N H_3PO_4 into 100 ml volumetric flask Bring up to 100 ml.

Pour into left sparging vial and then sparge.

Prepare blank:

MQ + approximately 1 ml (to pH < 2.0) 1N H_3PO_4. Sparge on right side.

Machine start-up:
The detector should be left on at all times; if it is off, turn on and allow at least a 2 h warm-up.

Turn on POWER, ASM; PUMP off, LAMP off, FURNACE on. Turn on the top box above FURNACE also (MANUAL). Open O_2 tank valve - regulator should read approximately 45 psi. The actual pressure showing on the gauge is not critical, but the flow rates through the instrument are critical. Unless one encounters a problem that justifies altering the flow, please leave the regulator as is.

Turn TOC/POC knob to TOC.

Turn range knob to 40 ul.

Change air flow configuration:

Connect "furnace air out" tube to "air in" port on side of UV reaction module. Connect "furnace air in" to "air out" port. Do this right after turning on furnace.

Allow approximately 30 min to 1 h for stabilization of baseline (0.0100 $\pm$ 0.0004) and furnace warm-up (a red glow is a rough indication of approximately 800 ^{0}C).

II. Calibration:

Place a small piece of quartz wool in a platinum boat that has been baked to burn off impurities (slide boat into furnace for 2 minutes or until detecter reading stabilizes). Inject 40 ul (using the yellow-top syringe) of 2000 ppm carbon standard into the boat, push START, then slide the boat into the furnace. Make sure that all air bubbles have been purged from the syringe before shooting into the boat. When the display indicates that the OC peak is on the downhill side, slide the boat out of the furnace and into flip top inlet block to cool. Inject another 40 ul of 2000 ppm standard. When READY signal beeps, push CALIB as quickly as possible momentarily*. Then run another 40 ul aliquot of standard. Analyzer printout should then read 2000 $\pm$ 40 ppm. Next run 40 ul of acidified blank that has been sparged. Remove quartz wool before running sediment samples.

*If printer reads 'NO CAL' then you have pressed CALIBRATE button too slowly. If so, then you must recalibrate by injecting two or more additional 2000 ppm standards.

III. To run samples:

1) Each OC sediment sample must be ground as fine as possible using a mortar and pestle.

2) Weigh out 0.01g dried and ground sample into a baked platinum boat. You may have to adjust sample size to get reading into 200-4000 ppm range.

3) Each time the door is opened to insert a boat, you must wait for the atmospheric CO_2 peak to clear on the detecter reading before running another sample.

4) Check baseline before sliding boat into furnace (should read 0.01).

4) Slide boat into furnace.

5) Push START.

6) When detector display begins downhill side of peak, pull boat out of furnace into flip top box so it can cool before inserting next sample.

7) Do not open flip top boat box until sample has run and the analyzer beeps!

To turn off analyzer:

a) Turn off furnace, ASM, and top box above furnace
b) Turn off gas

IV. To calculate % OC:

The carbon analyzer generates a number that is measured in mg/kg (ppm). This number is the Total Organic Carbon present in the sample. Use this number as [conc] in the equation below:

$$\%OC = \frac{\text{mass of carbon}}{\text{mass of sediment}} \times 100$$

$$\%OC = \frac{\text{conc (mg/l) (40ul) (1L/1 X } 10^6\text{ul) (1g/1 X } 10^3\text{mg)}}{\text{wt of sample (g)}} \times 100$$

$$\%OC = \frac{(conc)\ (40/1 \times 10^9)}{wt\ of\ sample} \times 100$$

Organic Carbon Procedure - Water Samples

Same as sediment procedure except:

Standards:
Make a 100 mg/L standard by taking 5.0 ml of the 2000 mg/L stock standard into a 100 ml volumetric flask with 1-2 ml 1N H_3PO_4 and bring to level with MQ water. This standard should read 10 mg/L when injected into the analyzer with a 1 ml syringe. This std should also read 100 mg/L on the auto-analyzer.

To turn on: Turn on POWER button on top box above furnace.

ASM: POWER on; PUMP on; LAMP on.

Open O_2 tank valve.

Range knob set at 1ml.

'Air in' and 'Air out' ports should be connected by the 'U' tube.

Calibrate with 10 mg/L std - everything else as usual.

Acidify each sample with 1ml H_3PO_4 before running; acidify blanks also.

Loading auto sampler:

1) Put first test tube next to rubber ring on the tray (to the left of the rubber ring).

2) First test tube should be a blank followed by: std, std, 3 more blanks, sample1, sample2, etc.

3) Use standard and 2 blanks midway through run.

4) At the end of the run use: std, 2 blanks.

REDOX POTENTIAL
(after Bohn, 1971 and Orion, 1983)

The oxidation-reduction potential (redox potential or Eh) is defined as the electromotive force developed by a platinum electrode immersed in a water or sediment sample relative to a standard hydrogen electrode or a reference electrode of known Eh. The obtained value is a crude estimate of the oxygen status of the sample. At pH 7.0, oxidized soils are at redox potentials of >400 mv, moderately reduced soils about 100 to 400 mv, reduced soils -100 to 100 mv, and highly reduced soils -100 to -300 mv.

Sample Collection and Storage

The preferred method of obtaining oxidation-reduction potential data is in situ measurement. If this is impractical, the measurements should be made as soon as possible. Since exposure to the atmosphere may affect the oxidation-reduction potential of the sample (oxygen may dissolve in water or oxidize sediments), precautions should be taken to minimize sample contact with the atmosphere prior to measurement of the oxidation-reduction potential. This precaution will necessitate the use of wet sediment samples for the measurement.

Calibration of Redox Electrode

1. Solution A:

   Weigh out: 4.22g reagent-grade $K_4Fe(CN)_6 * 3H_2O$ (potassium ferrocyanide)
   1.65g reagent-grade $K_3Fe(CN)_6$ (potassium ferricyanide)

   Place in a 100 ml volumetric flask. Add approximately 50 ml distilled water and swirl to dissolve crystals. Dilute to volume with distilled water.

2. Solution B:

   Weigh out: 0.42g potassium ferrocyanate
   1.65g potassium ferricyanate
   3.39g potassium fluoride ($KF * 2H_20$)

Place in erlenmeyer flask with screw cap, dissolve crystals and dilute to volume with distilled water.

3. Make sure Markson meter is on standby mode. Insert probe and adapter into GLASS and REF positions respectively on the back of the Markson ElectroMark Analyzer.

4. Place probe in solution A flask. Rinse probe with Milli Q water and gently wipe dry with Kimwipe. Place redox electrode in solution A and turn to mV. Wait until reading stabilizes. The potential should be approximately 234 mV. If not, turn STANDARDIZED knob until meter reads 234 mV. Lock standardize knob.

5. Rinse electrode with Milli Q water and repeat measurement with solution B. Reading should be approximately 300 mV. If not, turn STANDARDIZED knob until meter reads 300 mV.

6. After calibration is complete, rinse electrode with Milli Q water and place in sediment sample. Allow meter reading to stabilize. This could take 10-15 minutes.

7. After reading has stabilized, record reading in mV, remove probe from sample and rinse probe with MQ water to remove all sediment. Then blot dry with Kimwipe.

8. Repeat steps #6 and #7 for each subsequent sample.

9. After last sediment sample has been run, turn FUNCTION knob to STANDBY, rinse, dry and remove probe from meter.

APPENDIX C

ORGANISM CULTURE PROCEDURES

PROCEDURES FOR MAINTAINING IN-HOUSE *DAPHNIA MAGNA* CULTURES FOR USE IN TOXICITY TESTS

This procedure describes the methods for culturing *Daphnia magna* for use in toxicity tests. It is based on the methods described in Biesinger *et al.* (1987), and Peltier and Weber (1984).

DAPHNIA SOURCE

Daphnia were originally obtained from the U.S. Environmental Protection Agency, Environmental Monitoring Support Laboratory, Cincinatti, OH.

GENERAL PROCEDURE

Daphnia were maintained in 1000 ml borosilicate glass beakers containing 800 ml of filtered pond water from the University of North Texas Water Research Field Station (WRFS). New cultures were started once a week with 8 neonates (<48 h old) and 800 ml of culture water per beaker. Eight new beakers were started each week. The cultures were fed 2.5 mg/l of a yeast, trout chow, and alfalfa solution daily. The culture was maintained in a Precision Model 818 illuminated incubator ($21\pm2^{\circ}C$; 50-100 fc; 16h light/ 8h dark). The date of first neonate production was recorded for each beaker. Neonates were removed and 200 ml of water was renewed from the cultures every Monday, Wednesday, and Friday. At that time mortality, neonate production, water renewal, and presence of gravid females was recorded for each culture. Adults were discarded at the end of four weeks.

CULTURE WATER

Although reconstituted hard water is recommended by Biesinger *et al.* (1987), *D. magna* cultures were maintained using filtered pond water from the maintenance pond at the University of North Texas WRFS. Natural waters were used due to the presence of vitamins and nutrients which are not found in reconstituted hard water. Culture water was filtered though a Whatman EPM2000 filter to remove any particulate matter or suspended solids and to prevent contamination of the culture by other species of daphnids.

FOOD PREPARATION AND FEEDING

The YFA diet is a 500 ml solution consisting of baker's yeast (4.6 g/l), catfish chow (12.6 g/l), and alfalfa (1.0 g/l) and deionized water. The mixture was homogenized in a tissue blender for five minutes at high speed. The solution was then allowed to settle for one hour. After the solution had settled, the supernatant (top layer) was saved and decanted into plastic bottles in 30-50 ml aliquots and frozen until needed.

CULTURE CONDITIONS

Temperature:	21±2°C
Light Quality:	Incubator illumination (cool white light)
Light Intensity:	50-100 fc
Photoperiod:	16h light/8h dark

WATER CHEMISTRY

pH:	7.5-8.5
Hardness:	51-192 mg/l
Alkalinity:	120-178 mg/l
Dissolved Oxygen:	>40% of saturation

NEONATE REMOVAL (CULLING)

Separating the neonates from the adults is referred to as "culling". The adults were gently pipeted, using a large bore pipet (6mm ID), out of the culture beaker into a holding beaker. The holding beaker contained 50-100 mls of filtered pond water. Care was taken to release the adults below the surface of the water. The neonates in the beaker were gently poured into a culling chamber. The drain hose was unclamped and the culture water was drained back into the original culture beaker. The adults were then pipeted out of the holding beaker and placed back into the original culture beaker. Care was taken to release the adults below the surface of the water in the beaker. Culture water (200ml) was renewed at this point. Polystyrene petri dishes were placed over the beakers to prevent contamination and evaporation.

REMOVAL OF NEONATES FOR TOXICITY TESTS

Daphnia (3 days old) used for toxicity tests were individually pipeted into a beaker of filtered pond water. Pipetting by hand helped prevent floating that can sometimes occur when daphnids are transferred by pouring.

STARTING NEW CULTURES

New cultures were started weekly. Eight neonates <48 h old were placed in 800 ml of filtered pond water containing 5 ml of YFA solution. Eight beakers were started each week and were referred to as a "set of cultures" or "row". The date and the page number were written on each beaker to record the age of the daphnids.

ROUTINE MAINTENANCE

Each culture beaker was fed YFA diet daily. Neonates were culled and 200 ml of culture water was renewed from each culture beaker on Mondays, Wednesdays, and Fridays. Once per week, the sides of the beakers were wiped with a Kimpipe to remove algal growth and accumulated food. Daphnids were examined for the presence of the first brood. The date of the first brood was recorded in the D. magna culture production log. Neonates from the first brood were not used in tests or used to begin new cultures. Any individual culture beaker which showed mortality ≥10% per day was discarded. The daphnids were discarded after the fourth week. Date to first brood, culture number, temperature, DO, pH, conductivity, hardness, and alkalinity were measured weekly according to Standard Methods (1985).

PROCEDURES FOR MAINTAINING IN-HOUSE HYALELLA AZTECA CULTURES FOR USE IN TOXICITY TESTS

This procedure describes the methods for culturing Hyalella azteca for use in toxicity tests. It is based on the methods described in de March (1981).

HYALELLA SOURCE

Hyalella azteca (Saussure) were originally obtained from the U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Center, Columbia, MO.

CULTURE FACILITIES

Amphipod cultures were maintained in 10 gal glass aquaria filled with filtered, dechlorinated tap water. The cultures were in an isolated room, free of contamination and excessive disturbances. The cultures were maintained at a temperature of 22±3°C and a wide spectrum light intensity of approximately 100 f.c. in a 16h light, 8h dark regime. Cultures were vigorously aerated.

Note: If the amphipods come to the surface of the water or move up the side of the glass, the DO level is too low. Aeration should be increased.

CULTURE WATER

H. azteca cultures were maintained using filtered, dechlorinated tap water. Once per week, half of the water in each aquarium was siphoned off and replaced with clean, dechlorinated tap water.

SUBSTRATE

Hyalella feed on and live in maple leaves that are placed on the bottom of the culture aquaria. Fallen maple leaves (Acer spp.) were soaked in aged tap water for 10 days. The leaves were flushed to remove naturally occurring tannic acid before they were placed into the culture aquaria. Leaves were placed in the aquaria at a depth of approximately 5 cm. Additional leaves were added periodically when the amphipods had eaten most of the organic portions of the leaves.

FOOD PREPARATION AND FEEDING

Hyalella were fed ground Purina Rabbit Chow once per week. Rabbit chow was ground by mixing in a blender at high speed for 1 minute. Ground pellets were placed in an air-tight container and kept in a freezer until needed.

REMOVAL OF HYALELLA FOR USE IN TOXICITY TESTS

Amphipods were isolated by removing a part of the leaf mat from a culture aquarium using a 5" fine mesh net. This leaf mat was placed on a 1 mm (#18) mesh sieve and dechlorinated tap water was then gently poured over the leaves to flush the amphipods into the next sieve (#30) below. The Hyalella were washed through a #30 sieve (U.S. Standard series, 600 um) to obtain animals approximately 3-4 mm in length. The amphipods were then gently rinsed into a shallow stainless steel pan to be transferred to the test beakers. The organisms passing through the #18 sieve and trapped by the #30 sieve were used in the toxicity tests.

PROCEDURES FOR CULTURING THE MIDGE, CHIRONOMUS TENTANS FOR USE IN TOXICITY TESTS

PURPOSE AND SCOPE

Aquatic safety evaluations of chemicals require conducting toxicological studies with aquatic organisms in the laboratory. This procedure provides guidelines on culturing the midge, Chironomus tentans in the laboratory for support of a midge aquatic toxicity testing program.

BACKGROUND INFORMATION

It has been recognized that there is a need to include sediment dwelling aquatic organisms when testing chemicals that sorb to particulates and bottom sediments. Bioaccumulation studies are often a part of chemical safety evaluations and require organisms with a relatively large mass to be conducted conveniently. The midge, Chironomus tentans (Diptera:Chironomidae), a mosquito-like fly, is recognized as a useful test organism representing the aquatic benthic community in aquatic safety testing. The immature midge (larvae) is worm-like and lives in a case built within soft, flocculent sediments in a variety of aquatic habitats. Larvae can reach a size of 30 mm in length and 25 mg wet weight, making C. tentans one of the larger chironomids. C. tentans is easily cultured in the laboratory with readily available materials.

QUALITY OF CULTURES

Care was taken in culturing to insure healthy organisms were available for testing. This goal was met by carefully following the practices that have proven successful (listed below).

FACILITIES

Midges were cultured in an isolated area or room, free of contamination and excessive disturbances. Water was maintained at a temperature of 21° $\pm$ 2°C and a wide spectrum light intensity of approximately 100 f.c. in a 16 hour light, 8 hour dark regime.

CULTURE WATER

C. tentans were cultured in filtered and declorinated tap water.

CULTURE CHAMBERS

Midges were cultured in 5 to 10 gallon glass aquaria filled with dechlorinated tap water to a depth of 8 cm. Culture chambers were covered with polyethylene (Saran Wrap) or a screen to prevent adults from escaping and to exclude other species from entering the cultures. Culture chambers were vigorously aerated with an airstone to maintain a satisfactory dissolved oxygen (DO) concentration.

Note: If the midges come to the surface of the substrate or move up the side of the glass, the DO level is too low. Aeration should be increased.

MAINTENANCE

Approximately half of the culture water in each aquarium was changed weekly by using a siphon hose to eliminate accumulated waste products. While the water level was down, a razor blade was used to scrape the sides of the aquarium to remove fungus and algae. Care was taken to avoid siphoning too much water before cleaning. Fresh water was added slowly to prevent excessive turbulence. Pupal casings, dead adults, etc. were picked out on a regular basis in order to maintain water quality between water changes.

MIDGE SOURCE

The midges used in the UNT Water Research Field Station Aquatic Toxicology Lab were obtained from the Monsanto Co, St. Louis, Mo.

SUBSTRATE

C. tentans requires a substrate in which to construct a case. Brown shredded paper towels have been found to be well suited for this purpose. Bleached towels are not a good substrate. Strips cut from #1052.5 Industrial Crown-Zellerback (Nibroc) brown paper towels were soaked overnight in acetone to remove impurities. The towels were then boiled in three changes of tap water until all acetone was removed. A tissue blender was then used to shred the towels into a pulp. Care was taken to avoid overblending and

possible shortening of the wood fibers in the pulp. Before being added to the culture, the paper towels were rinsed twice with dechlorinated tap water to remove extremely small fibers. The paper pulp was placed into the water of a culture chamber until a depth of 3 cm was obtained. Extra clean paper pulp material was refrigerated until needed. From time to time, new pulp was added to established cultures to replace used pulp.

FOOD PREPARATION AND FEEDING

C. tentans is primarily a filter feeder drawing food particles into its case from the water column. A suspension of "Tetra Conditioning Food Vegetable Diet" has been used with good success. Fifty grams of fish flake was added to 500 ml of dechlorinated tap water and mixed with a tissue blender for 5 minutes. The mixture was then sieved through a #230 stainless steel sieve. The mixture was allowed to settle overnight. The midges were fed the upper layer only as the bottom layer tends to be high in BOD's. Extra food was frozen until needed. Portions in use were refrigerated between feedings. For optimal growth of the culture, larvae were fed daily. The midges were fed 1.0 ml of the Tetra suspension twice per day. If the water was not clear in 3 or 4 hours after feeding, too much food was added. Overfeeding will lead to the growth of fungi in the aquaria and will necessitate more frequent water changes.

MIDGE LIFE CYCLE

C. tentans egg masses hatch in 2 to 3 days after deposition in water at temperatures of 19 to 22°C. Larval growth occurs in 4 instars of approximately one week each. Under optimal conditions, some larvae will develop into adults 24 to 28 days after egg deposition. Adults emerge from pupal cases over a period lasting several weeks. Adult males are easily distinguished from females in that they have large, plumose antennae and a much thinner abdomen with visible genetalia. Egg masses are removed from the tanks four days before larvae are needed for testing and placed in 20 ml of aged, filtered and dechlorinated tap water. Eggs can be stored in a refrigerator to retard development but after 4 or 5 days, viablity is greatly reduced.

CULTURE LOGISTICS

Each egg mass contains from 300 to 500 eggs. Two or three egg masses laid gently on the substrate is enough to start a culture chamber. A culture chamber may be productive for several months and can be expected to produce a few adults each day once generations of larvae are staggered. Once a culture becomes unproductive because of worn out substrate or contamination by other detrimental organisms, it should be disposed of and a new culture started. Several cultures of different ages should be maintained at any one time as a hedge against unfavorable occurances.

APPENDIX D

ORGANISM TESTING PROCEDURES

PROCEDURES FOR CONDUCTING STATIC CHRONIC WHOLE SEDIMENT TOXICITY TESTS WITH *DAPHNIA MAGNA*

SCOPE

This procedure describes the methods used by the UNT Water Research Field Station (WRFS) Aquatic Toxicology Laboratory for obtaining laboratory data to evaluate adverse effects of contaminants in whole sediment tests. This method is designed to assess the toxic effects of fluoranthene on *Daphnia magna* survival in 10 day whole sediment exposures in static systems. Modification of these procedures may be justified by special needs. Comparison of results obtained using modified versions of these procedures might provide useful information on new concepts and procedures for conducting toxicity tests with *Daphnia magna*.

This document is to be viewed as a supplement to Peltier and Weber (1985), and Beisinger, *et al*. (1987).

FACILITIES

Tests were conducted in temperature and light controlled incubators (Precision Model 818). Temperature was maintained at 20 $\pm 1^{\circ}C$ with a 16h light, 8h dark photoperiod.

TEST CHAMBERS

Tests were conducted in 250 ml borosilcate glass beakers and covered with polystyrene petri dishes to prevent airborn contamination.

FILTRATION

Test water was vacuum filtered through Whatman EPM2000 filters to remove large particles, suspended solids, and contaminant organisms naturally occurring in the pond water.

CLEANING

Test beakers and other equipment which came into contact with fluoranthene (Flu) was properly washed before each use. All glassware and equipment was first rinsed with deionized

water followed by one rinse with reagent grade acetone. Final rinse was with HPLC grade hexane. All glassware was dried thoroughly before use.

DILUTION WATER

Dilution water was uncontaminated and of consistant quality. All water used in testing was obtained from the WRFS maintenance pond.

CHARACTERIZATION

Dissolved oxygen, pH, hardness, alkalinity, and conductivity were measured on day 0 and day 10 of each definitive test. Dissolved oxygen was measured more frequently if <40% of saturation.

TEST ORGANISMS

All daphnids were as uniform as possible in age and size class. D. magna neonates (3 days old) were used in the toxicity tests.

DAPHNIA SOURCE

All daphnids used in testing were from the same source. Daphnia used in WRFS tests were obtained from in-house cultures.

EXPERIMENTAL DESIGN

Screening Tests
Screening tests were used to determine whether or not the sediments exhibited background toxicity to the daphnids. Five replicates (beakers) were used with ten Daphnia per replicate. Test duration was 10 days. Each screening test was conducted as closely as possible to the definitive test conditions to reduce variability.

Range-Finding Tests
Range-finding tests were used to determine the range of concentrations of Flu to be used for the definitive tests because little was known of the toxicity of fluoranthene (Flu). Range-finding tests had five or more concentrations spanning one or more orders of magnitude. There was only one

replicate and ten daphnids per concentration. Test duration was 10 days. Test conditions were the same as for the definitive tests.

Definitive Tests
The definitive tests consisted of a sample of daphnids exposed for 10 days to a series of Flu concentrations. These tests were used to estimate the EC50 which was the Flu concentration required to produce an effect (mortality) to 50% of the daphnids at the end of 10 days. Test vessels were 250 ml borosilicate glass beakers. Six sediment concentrations and a control were used per sediment with three replicates per concentration.
The sediment was homogenized first with a power drill and stainless steel impeller. Forty ml of wet sediment was measured in a 50 ml beaker and placed into a 250 ml test beaker. The test beakers were gently shaken to facilitate settling of the sediment. For range-finding and definitive tests, the fluoranthene was added to the sediment as described in Appendix F. After sediment spiking was completed, 160 ml of WRFS pond water was carefully added by tilting the beaker and pouring the pond water down the side of the beaker. Test beakers were left to settle overnight before adding Daphnia (this is Day -1). The following day, ten daphnids were placed gently into each beaker (this is Day 0). After adding the daphnids to the test beakers, the beakers were covered with polystyrene petri dishes. Each beaker contained 40 ml of wet homogenized sediment and 160 ml of WRFS pond water. Test beakers were not aerated during the test.

Test Termination
After the water samples were removed from the test beakers, the overlying water containing the daphnids was poured through a 500 um mesh stainless steel sieve. The Daphnia were large enough to be trapped by the sieve and were then easily counted.

ANALYSES

WATER ANALYSIS

A 3 ml sample of the overlying water was extracted from each test beaker in range-finding and definitive tests on Day 0 and Day 10 and analyzed on a spectrophotofluorometer (SPF) as described in Appendix E.

SEDIMENT ANALYSIS

A 1.00 gram sample of sediment was extracted from each test beaker on Day -1 and Day 10 in range-finding and definitive tests and analyzed on an SPF as described in Appendix E.

INTERSTITIAL WATER ANALYSIS

For definitive tests only:
On day 10, all the sediment from one replicate of each concentration was placed in a 40 ml centrifuge tube and centrifuged for 10 minutes at 300 (xg) and subsequently analyzed on an SPF as described in Appendix E.

TEST ENDPOINTS

The endpoint for this study was survival after 10 days. Total neonate production was also recorded at Day 10.

WATER CHEMISTRY

Temperature
The temperature range for testing was 20±2oC. Temperature was monitored daily.

DO did not drop below 40 percent of saturation except in Trinity River tests. Measurements were made at the beginning of the test and as needed thereafter if DO <40 % of saturation. Conductivity, pH, alkalinity, hardness were measured at the beginning of the test and at test termination.

FEEDING

D. magna were fed 2 drops of the YFA diet on days 0, 2, 4, 6, and 8.

AERATION

No aeration was used during the study.

TEST ENDPOINTS

EC50 - Immobility was defined as the inability of the daphnids to swim or hop off the bottom of the beaker under its own power. Daphnids were observed for 10 seconds.

PROCEDURES FOR CONDUCTING STATIC CHRONIC WHOLE SEDIMENT TOXICITY TESTS WITH HYALELLLA AZTECA

SCOPE

This procedure describes the methods used by the UNT Water Research Field Station (WRFS) Aquatic Toxicology Laboratory for obtaining laboratory data to evaluate adverse effects of contaminants in whole sediment tests. This method is designed to assess the toxic effects of fluoranthene (Flu) on Hyalella azteca survival in 10 day whole sediment exposures in static systems. Modification of these procedures may be justified by special needs. Comparison of results obtained using modified versions of these procedures might provide useful information on new concepts and procedures for conducting toxicity tests with Hyalella azteca.

FACILITIES

Tests were conducted in temperature and light controlled incubators (Precision Model 818). Temperature was maintained at $20\pm1^{\circ}C$ with a 16h light, 8h dark photoperiod.

TEST CHAMBERS

Tests were conducted in 250 ml borosilicate glass beakers and covered with polystyrene petri dishes to prevent airborn contamination.

FILTRATION

Test water was vacuum filtered through Whatman EPM2000 filters to remove particles, suspended solids, and contaminant organisms naturally occurring in the pond water.

CLEANING

Test beakers and other equipment which came into contact with Flu was properly washed before each use. All glassware and equipment was first rinsed with deionized water followed by one rinse with reagent grade acetone. Final rinse was with HPLC grade hexane. All glassware was dried thoroughly before use.

DILUTION WATER

Dilution water was uncontaminated and of consistant quality. All water used in testing was obtained from the WRFS maintenance pond.

CHARACTERIZATION

Dissolved oxygen, pH, hardness, alkalinity, and conductivity were measured on day 0 and day 10 of each definitive test. DO was measured daily if below 40% saturation.

TEST ORGANISMS

All amphipods were as uniform as possible in age and size class. The largest amphipods to pass through a 1mm mesh stainless steel screen but fail to pass through a 600 um mesh stainless steel screen were used for testing.

SOURCE

All Hyalella used in testing were obtained from in-house cultures.

EXPERIMENTAL DESIGN

Screening Tests
Screening tests were used to determine whether or not the sediments exhibited background toxicity to the amphipods. Five replicates (beakers) were used with ten amphipods per replicate. Test duration was 10 days. Each screening test was conducted as closely as possible to definitive test conditions to reduce variability.

Range-Finding Tests
Range-finding tests were used to determine the range of concentrations of Flu to be used for the definitive tests because little was known of the toxicity of Flu. Range-finding tests had five or more concentrations spanning one or more orders of magnitude. There was only one replicate and five amphipods per concentration. Test duration was 10 days. Test conditions were the same as for the definitive tests.

Definitive Tests
The definitive tests consisted of a sample of amphipods exposed for 10 days to a series of Flu concentrations. These tests were used to estimate the EC50 which was the Flu concentration required to produce an effect (mortality) to

50% of the Hyalella at the end of 10 days. Test vessels were 250 ml borosilicate glass beakers. Six sediment concentrations and a control were used per sediment with three replicates per concentration.

The sediment was homogenized first with a power drill and stainless steel impeller. Forty ml of wet sediment was measured in a 50 ml beaker and placed into a 250 ml test beaker. The test beakers were gently shaken to facilitate settling of the sediment. For range-finding and definitive tests, the Flu was added to the sediment as described in Appendix F. After sediment spiking was completed, 160 ml of WRFS pond water was carefully added by tilting the beaker and pouring the pond water down the side of the beaker. Test beakers were left to settle overnight before adding the amphipods (this is day -1). The following day, ten Hyalella were placed gently into each beaker (this is day 0). After adding amphipods to the test beakers, the beakers were covered with polystyrene petri dishes. Each beaker contained 40 ml of wet homogenized sediment and 160 ml of WRFS pond water. Test beakers were not aerated during the test.

Test Termination
After the water samples were removed from the test beakers, the overlying water containing the amphipods was poured through a 500 um mesh stainless steel sieve. Amphipods were large enough to be trapped by the sieve and were then easily counted.

WATER CHEMISTRY

Temperature
The temperature range for testing was $20 \pm 1^{\circ}C$. Temperature was monitored daily.

DO did not drop below 40 percent of saturation except in Trinity River tests. Measurements were made at the beginning of the test and as needed thereafter if DO was below 40% saturation.
Conductivity, pH, alkalinity, hardness were measured at the beginning of the test and at test termination.

WATER ANALYSIS

A 3 ml sample of the overlying water was extracted from each test beaker in range-finding and definitive tests on Day 0 and Day 10 and analyzed on a spectrophotofluorometer (SPF) as described in Appendix E.

SEDIMENT ANALYSIS

A 1.00 gram sample of sediment was extracted from each test beaker on Day -1 and Day 10 in range-finding and definitive tests and analyzed on an SPF as described in Appendix E.

INTERSTITIAL WATER ANALYSIS

For definitive tests only:
On Day 10, all the sediment from one replicate of each concentration was placed in a 40 ml centrifuge tube and centrifuged for 10 minutes at 300 (xg) and subsequently analyzed on an SPF as described in Appendix E.

FEEDING

Amphipods were fed 0.001g of ground Purina Rabbit Chow on Day 0 and Day 5 of the test.

AERATION

No aeration was used during the tests.

TEST ENDPOINTS

EC50 - Mortality. Mortality was defined as no amphipod movement when sieved at test termination (healthy amphipods move quite rapidly when poured on a sieve). Dead Hyalella usually curl up in the shape of a half moon and turn a whitish color when dead.

PROCEDURES FOR CONDUCTING STATIC CHRONIC WHOLE SEDIMENT TOXICITY TESTS WITH THE MIDGE, CHIRONOMUS TENTANS

PURPOSE AND SCOPE

This sediment testing method is a guideline for testing sediments with midges in a static system. The principal objective is to assess potential toxicity of contaminants associated with sediments collected from the field or toxicant spiked in the laboratory. Procedures are described for obtaining laboratory data concerning the chronic effect of contaminants associated with the solid phase of sediment to midges.

TEST ORGANISM

Chironomus tentans Fabricius (Chironomidae, Diptera) is the recommended test species due to its ease of culturing, relatively large size as second instar larvae, short time required to raise larvae to second instar, and ease of handling the larvae. This species is widely distributed throughout the U.S. and spends the larval portion of its life cycle in a tunnel or case within the upper sediment layer of lakes, rivers and estuaries. Its feeding habits include both filter feeding and ingesting sediment.

PROCEDURES

Preliminary Preparations

The life cycle of C. tentans takes approximately 24-28 days to complete at 25°C. Adult midges are collected in 250 ml flasks where they mate overnight. Egg masses, each containing 300-500 eggs, are isolated in 50 ml beakers containing dechlorinated tap water following deposition. The length of time from oviposition to hatch is approximately 3-4 days at 23°C. On Day 4 newly hatched larvae are transferred to isolation chambers (12x13x20cm) containing shredded paper towel substrate, Tetra Conditioning Food and dechlorinated tap water which is aerated through a Pasteur pipet. By Day 10 the larvae have reached the second instar stage of development and are ready for use in sediment tests. To remove the midges and bring them to the surface of the isolation chamber, stir vigorously using a circular motion with a spatula or hollow glass pipet.

TEST FACILITIES

Tests were conducted in temperature and light controlled incubators (Precision Model 818). Temperature was maintained at 20±1°C with a 16h light, 8h dark photoperiod.

TEST WATER

Test water was uncontaminated and of consistant quality. All water used in testing was obtained from the WRFS maintenance pond.

EXPERIMENTAL DESIGN

Screening Tests

Screening tests were used to determine whether or not the sediments exhibited background toxicity to the midges. Five replicates (beakers) were used with five midges per replicate. Test duration was 10 days. Each screening test was conducted as closely as possible to the definitive test conditions to reduce variability.

Range-Finding Tests

Range-finding tests were used to determine the range of concentrations of fluoranthene (Flu) to be used for the definitive tests because little was known of the toxicity of Flu. Range-finding tests had five or more concentrations spanning one or more orders of magnitude. There was only one replicate and five midges per concentration. Test duration was 10 days. Test conditions were the same as for the definitive tests.

Definitive Tests

The definitive tests consisted of a sample of midges exposed for 10 days to a series of Flu concentrations. These tests were used to estimate the EC50 which was the Flu concentration required to produce an effect (mortality) to 50% of the midges at the end of 10 days. Test vessels were 250 ml borosilicate glass beakers. Six sediment concentrations and a control were used per sediment with three replicates per concentration.

The sediment was homogenized first with a power drill and stainless steel impeller. Forty ml of wet sediment was measured in a 50 ml beaker and placed into a 250 ml test beaker. The test beakers were gently shaken to facilitate settling of the sediment. For range-finding and definitive tests, the Flu was added to the sediment as described in Appendix F. After sediment spiking was completed, 160 ml of

WRFS pond water was carefully added by tilting the beaker and pouring the pond water down the side of the beaker. Test beakers were left to settle overnight before adding the midges (this is Day -1). The following day, five chironomids were placed gently into each beaker (this is Day 0). After adding midges to the test beakers, the beakers were covered with polystyrene petri dishes. Each beaker contained 40 ml of wet homogenized sediment and 160 ml of WRFS pond water. Test beakers were not aerated.

Test Termination
After the water samples were removed from the test beakers, the overlying water was poured through a 500 um mesh stainless steel sieve. If the midges were still in the sediment, the top layer of sediment was rinsed with a squirt bottle containing deionized water (DI) and poured through the sieve. This procedure was repeated until all midges were removed from the sediment. Midges were large enough to be trapped by the sieve and were then easily counted.

FEEDING
Midges were fed 2 drops of Tetra Conditioning Food suspension every other day starting with Day 0.

TEST ENDPOINTS
The endpoint for this study was survival after 10 days. Mortality was defined as complete immobilization and lack of body movement upon gentle prodding.

WATER CHEMISTRY

Temperature
The temperature range for testing was 20±1°C. Temperature was monitored daily.

DO should not drop below 30 percent of saturation. If so, it was monitored daily. Measurements were made at the beginning of the test and as needed thereafter. Conductivity, pH, alkalinity, and hardness were measured at the beginning of the test and at test termination.

WATER ANALYSIS

A 3 ml sample of the overlying water was extracted from each test beaker in range-finding and definitive tests on Day 0

and Day 10 and analyzed on a spectrophotofluorometer (SPF) as described in Appendix E.

SEDIMENT ANALYSIS

A 1.00 gram sample of sediment was extracted from each test beaker on Day -1 and Day 10 in range-finding and definitive tests and analyzed on an SPF as described in Appendix E.

INTERSTITIAL WATER ANALYSIS

For definitive tests only:
On Day 10, all the sediment from one replicate of each concentration was placed in a 40 ml centrifuge tube and centrifuged for 10 minutes at 300 (xg) and subsequently analyzed on an SPF as described in Appendix E.

APPENDIX E

SEDIMENT SPIKING PROCEDURE USING FLUORANTHENE

SEDIMENT SPIKING PROCEDURE USING FLUORANTHENE

1) In a 50 ml volumetric flask, add 0.500 g of fluoranthene (Flu) (Aldrich Chemical Co.; Cat. #F80-7) and bring to volume with optima grade acetone. Invert several times to mix. This makes a 10,000 mg/L Flu stock solution that will be used to spike the sediments.

Note: 1 ul of stock solution = 10 ug of Flu

2) Once 40 ml of sediment was placed in the test beakers, the sediment was ready to be spiked. The Flu - acetone mixture was added to the wet sediment by a ul syringe (Hamilton Co., Reno Nevada). Since very little acetone was added to the sediment, the acetone evaporated very quickly upon contact with the sediment (within 3 min.) and left behind crystalline Flu which could then be mixed.

3) Sediment and Flu then mixed for approximately 1 min. using a stainless steel spatula.

4) After mixing, the Day -1 sediment sample was taken and then the overlying water was added.

APPENDIX F

SPECTROPHOTOMETRIC ANALYSIS OF FLUORANTHENE

AMINCO-BOWMAN

MODEL # 4-8202 SPF

SPECTROPHOTOFLUOROMETER (SPF)

FLUORANTHENE ANALYSIS

IN WATER

Note: This procedure is a condensed version of the Aminco-Bowman (June, 1976) SPF operator's manual. Consult manual for further information on the operation of this instrument.

A. Turning SPF on:

1. Turn switch on back of SPF on. Make sure blower comes on.

2. Turn xenon lamp power supply switch on, wait 20 sec., then push red starter button and hold about 2 sec. (You can look at the back of the SPF and see light coming on.)

3. Turn on ratio photometer by pushing power on button.

4. Turn H.V. control to manual, adjust current to 0.75 max (knob next to H.V. control).

<u>Allow SPF to warm up for 30 min.</u>

B. To zero the SPF.

1. Place blank (solvent) in a 1 cm x 2 cm quartz glass cuvette. Be sure to use the solvent the standards are dissolved in as a blank. Fluoranthene standards will use hexane as a solvent. (Make sure emission shutter is closed before removing the cell chamber cover. The rod should be in the up position.)

2. Turn sensitivity to zero adjust. Open emission shutter (down position).

3. Adjust to zero with zero adjust knob.

4. Turn sensitivity to 0.1.

5. With blank subtract on low, adjust to zero with the inner knob on the blank subtract knob.

6. Repeat steps 3, 4, 5 several times until the meter stays on zero.

C. Setting full scale to scan for unknown samples.

1. Close emission shutter and replace blank with standard.

2. Open emission shutter.

3. Using the excitation dial, select an excitation wavelength on the lower end of the scale.

* When setting excitation and emission wavelengths, always turn the dial to a setting above the desired wavelength and then slowly turn back.

4. Set the sensitivity switch to 3.

5. Place chart paper on chart recorder and release pen.

6. Turn monochromator switch to emission. (The instrument will scan all emission wavelengths for a response.)

* Be sure the pen lines up on the chart paper with the settings on the emission wavelength dial. Adjust the horizontal control on the chart recorder while the dial is moving.

7. Once the instrument has scanned the full range of wavelengths, the pen will return to the left side of the chart paper. At this point, turn the monochromator switch to off.

8. Increase the excitation wavelength by 100 and repeat steps 6 and 7.

9. Repeat step 8 until you bracket the response curve.

10. The excitation wavelength is the setting on the excitation dial that gave the greatest response.

11. The emission wavelength is the setting on the emission dial that gave the greatest response.

12. Set the instrument and the excitation and emission wavelengths desired.

13. With the sensitivity vernier adjust meter to 100 relative intensity. (If 100 relative intensity can be reached, lower sensitivity.)

Note: Always remember to close emission shutter when the cell chamber is open and to open the emission shutter when taking a measurement.

D. Setting scale for known samples.

1. Close emission shutter and replace blank with highest concentration standard.

2. Open emission shutter.

3. Using the excitation dial, select the excitation wavelength for the known sample. Fluoranthene has an excitation wavelength of 354 nm.

* When setting excitation and emission wavelengths, always turn the dial to a setting above the desired wavelength and then slowly turn back.

4. Using the emission dial, select the excitation wavelength for the known sample. Fluoranthene has an emission wavelength of 464 nm.

5. With the sensitivity vernier adjust meter to 100 relative intensity. (If 100 relative intensity can be reached, lower sensitivity.)

Note: Always remember to close emission shutter when the cell chamber is open and to open the emission shutter when taking a measurement.

6. Repeat steps 1 through 4 using lower standards and record relative intensity for each to establish a curve. Do not move the sensitivity vernier once it is set with the highest concentration standard.

E. READING SAMPLES

1. Place sample in cuvette.

2. Open emission shutter.

3. Read relative intensity.

4. Close emission shutter.

5. Remove sample.

Note: To determine concentrations of samples, a standard curve must be established.

F. To shut SPF off.

1. Turn H.V. control off.

2. Turn ratio photometer off.

3. Turn xenon lamp switch off.

4. After light has been off for about 2 min., turn SPF off.

G. Precautions

1. Use H.V. on manual only

2. Make sure shutter is closed when taking readings.

3. Don't look at light in cell when using the U.V.

4. Don't change any slit or mirror settings unless you know how and why. If you do change settings, return them to the way you found them when you are finished with samples.

5. Select a solvent which is invisible at the excitation and emission wavelengths selected.

H. Fluoranthene standards.

1. Fluoranthene (98%) stock is obtained from Aldrich Chemical Company (Cat # F80-7).

2. HPLC grade hexane is used as a solvent.

3. Standards of 200, 150, 100, 50, and 10 ug/L are prepared and stored in amber bottles at room temperature.

4. New standards should be prepared monthly.

I. Fluoranthene standard curve

1. A standard curve must be established using the 200, 150, 100, 50, and 10 ug/L standards. Standards are measured according to the procedure listed in section E.

2. Plot a regression of the actual intensity (AI) of each standard versus nominal concentration of the standard.

3. Actual intensity is calculated by multiplying the relative intensity (RI)(taken directly from the ratio photometer) by the sensitivity setting (SENS). Actual intensity is then multiplied by the slope of the standard curve plus the Y intercept of the line to obtain the concentration of FLU in ug/L:

   RI x SENS x SLOPE + Y INTERCEPT = CONC FLU (ug/L)

4. The slope of the line is calculated by entering the nominal FLU concentrations as 'Y' values and entering the corresponding actual intensities as the 'X' values on a calculator or PC BASS linear regression program.

5. A typical example follows:

NOM. CONC.	RI	x	SENS	=	AI	x	SLOPE	=	ug/L
200ppb	100		0.3		30		6.67		200
150	75		0.3		22.5		6.67		150
100	50		0.3		15		6.67		100
50	25		0.3		7.5		6.67		50
10	5		0.3		1.5		6.67		10

The slope for the above situation is 6.67 and the Y intercept is zero.

*Note: If the hexane/test water ratio is not 1:1 (eg. 6 ml pond water and 3 ml of hexane) then this must be accounted for in calculating the fluoranthene concentration in the following manner:

$$RI \times SENS \times AI \times SLOPE + INTERCEPT \times \frac{\text{mls hexane}}{\text{mls water}} = \text{ug/L FLU}$$

AQUEOUS PHASE FLUORANTHENE EXTRACTIONS FOR USE ON A SPECTROPHOTOFLUOROMETER (SPF)

1) Pipet out 3.0 ml of overlying water from the test vessel (250 ml beaker) into a 16 x 150 mm test tube with a teflon lined screw cap.

2) Filter each 3 ml sample with a Whatman EPM2000 filter using a 20 ml syringe and Millipore 2 Swinnex-13 filter holder.
Filter paper can be cut to size by using a 13 mm cork borer.
One filter can be used for all replicates within a concentration (usually 3 to 5 reps).

3) After filtering, add 3.0 ml HPLC grade hexane to each test tube.

4) Screw cap on tightly and then mix each sample on a vortex mixer (Thermolyne Type 16700) for 30 seconds.

5) Let tubes set for a few minutes to allow layers to fully separate.

6) To run samples on SPF:
Remove hexane (top layer) from the test tube with a pasteur pipet and place into the cuvette.

*Note: By filtering the pond water sample as in Step 2 above you remove the particulate matter in the water. Therefore the amount of FLU that is read on the SPF would be the total amount of <u>free</u> FLU in the water and not the total amount of FLU. The free FLU is the amount that the organism comes in contact with in the water column.

INTERSTITIAL (PORE) WATER FLUORANTHENE EXTRACTIONS FOR USE ON A SPECTROPHOTOFLUOROMETER (SPF)

1) Upon test completion, remove all test organisms and overlying water from the test beaker. With a large blade spatula, remove all the sediment from the beaker into a 40 ml centrifuge tube. Tap the tube gently on a counter top to ensure complete sediment settling in the tube.

2) Centrifuge sediment for 10 minutes at 300 (xg) (1600 RPM's) to obtain approximately 3 to 4 mls of pore water.

3) Carefully pipet out 2 ml of the pore water and place into a 20 ml test tube.

4) Filter each 2 ml sample with a Whatman EPM2000 filter with a filter holder and 20 ml syringe.

5) Screw on test tube cap tightly and vortex mix for 30 seconds.

6) Let test tubes set for a few minutes to allow layers to fully separate.

7) To run samples on SPF:

   Remove hexane (top layer) from the test tube with a pasteur pipet and place in the SPF cuvette. SPF procedures are the same as for overlying water.

Note: By filtering the pore water samples as in Step 4 above, you remove the particulate matter in the water. Therefore the amount of fluoranthene (Flu) that is read on the SPF would be the total amount of free Flu in the water and not the total amount of Flu. The free Flu is the amount that the organism comes in contact with in the water column.

AMINCO-BOWMAN

MODEL # 4-8202 SPF

SPECTROPHOTOFLUOROMETER (SPF)

FLUORANTHENE ANALYSIS

IN SEDIMENTS

Sediment procedures are identical to water procedures. Please see above method for water analysis.

H. Fluoranthene standards.

1. Fluoranthene (98%) stock is obtained from Aldrich Chemical Company (Cat # F80-7).

2. HPLC grade hexane is used as a solvent.

3. Standards of 200, 150, 100, 50, and 10 ug/L are prepared and stored in amber bottles at room temperature.

4. New standards should be prepared monthly.

I. Fluoranthene standard curve

1. A standard curve must be established using the 200, 150, 100, 50, and 10 ug/L standards. Standards are measured according to the procedure listed in section E.

2. Plot a regression of the actual intensity (AI) of each standard versus nominal concentration of the standard.

3. Actual intensity is calculated by multiplying the relative intensity (RI)(taken directly from the ratio photometer) by the sensitivity setting (SENS). Actual intensity is then multiplied by the slope of the standard curve plus the Y intercept of the line to obtain the concentration of FLU in ug/L. Then the sediment wet weight (grams), mls of hexane extract and sediment fraction solids must be considered. If the sediment extract does not fall within the standard curve, then the extract must also be diluted:

$$RI \times SENS \times SLOPE + Y\ INTERCEPT \times \frac{mls\ EXTRACT}{g\ SED\ WET\ WT} \times$$

$$\frac{mls\ OF\ HEXANE\ IN\ TEST\ TUBE}{AMOUNT\ EXTRACT\ REMOVED\ (mls)}\ (DILUTION\ STEP) \times$$

$$\frac{1}{FRAC\ SED\ DRY\ WT} = ug/kg\ FLU\ SEDIMENT\ DRY\ WEIGHT$$

*Note: If the sample recovery is not 100% and the actual percent recovery is known, then the final concentration of FLU should be divided by the fractional recovery. For example, if the percent recovery is 50%, then you should divide the final FLU concentration by 0.50.

4. The slope of the line is calculated by entering the nominal FLU concentrations as 'Y' values and entering the corresponding actual intensities as the 'X' values on a calculator or PC BASS linear regression program.

5. A typical example follows:

NOM. CONC.	RI	x	SENS	=	AI	x	SLOPE	=	ug/L
200ppb	100		0.3		30		6.67		200
150	75		0.3		22.5		6.67		150
100	50		0.3		15		6.67		100
50	25		0.3		7.5		6.67		50
10	5		0.3		1.5		6.67		10

The slope for the above situation is 6.67 and the Y intercept is zero.

SEDIMENT FLUORANTHENE

EXTRACTIONS FOR USE ON A

SPECTROPHOTOFLUOROMETER (SPF)

1) From the test vessel (250 ml beaker) remove 1.0 g of wet sediment and place in a 50 ml beaker.

2) To the 50 ml beaker containing the sediment add 10.0 ml of HPLC grade hexane.

3) Hand stir sediment/hexane mixture for 1 minute with a spatula or other appropriate instrument.

4) Place beaker into an ultrasonic cleaner (Bransonic Model 220) and sonicate for 3 minutes.

5) Decant hexane into a 20 ml test tube with a teflon lined screw cap.

6) Repeat Steps 3-5 for a second sediment extraction (total hexane extract = 20 ml).

7) Read sample on SPF.

*Note: The concentration of FLU obtained by this method is the total amount of FLU in the sediment.

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