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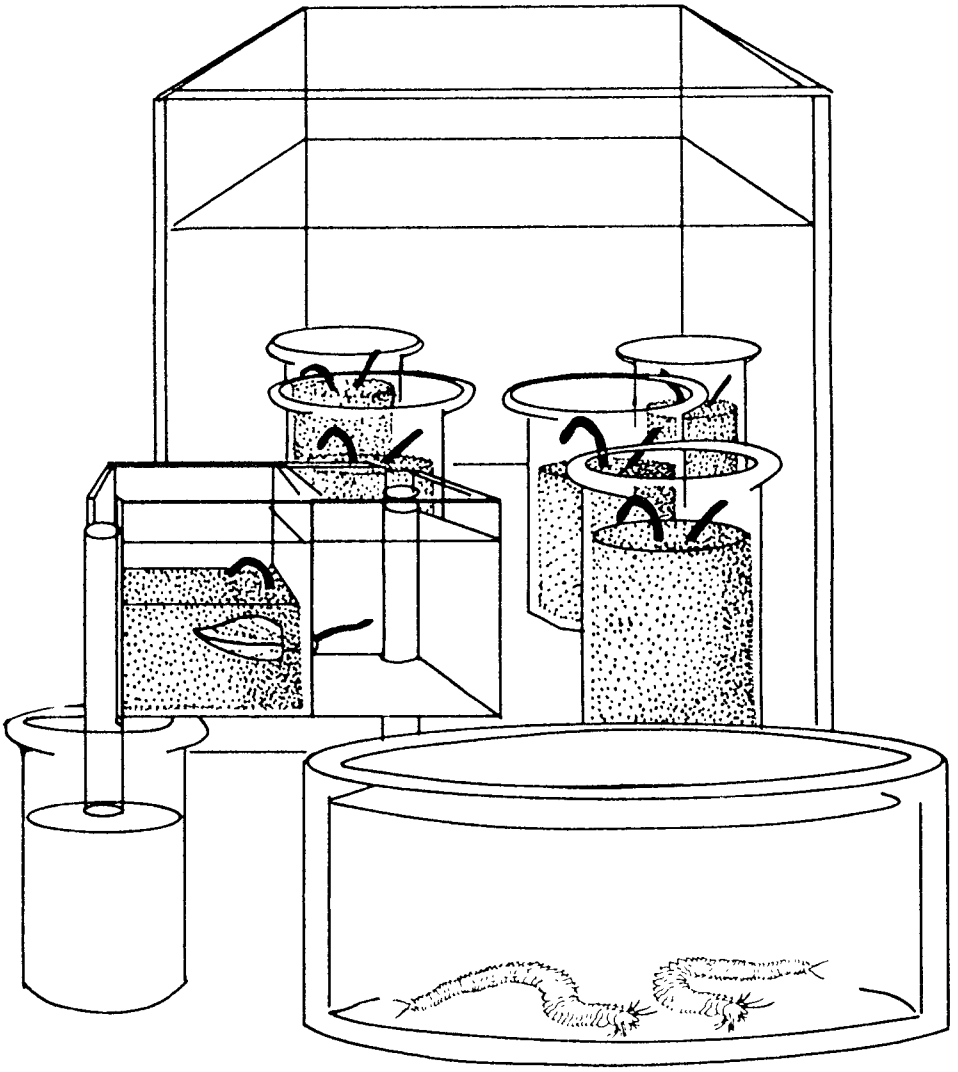
Guidance Manual

Bedded Sediment Bioaccumulation Tests



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GUIDANCE MANUAL: BEDDED SEDIMENT BIOACCUMULATION TESTS

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PREFACE

The recommendations in this document are based on our experience and best scientific judgment. It is our hope that the procedures suggested here will aid scientists in conducting "routine" and experimental sediment bioaccumulation tests, as well as aid regulators in determining when bioaccumulation tests are needed, in evaluating the QA/QC procedures of such tests, and in interpreting the results. The recommendations made here, however, do not constitute an official policy or standard procedure by the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation of use by the Environmental Protection Agency.

1993 UPDATE TO "BIOACCUMULATION GUIDANCE MANUAL"

In the four years since we wrote this document, the "Guidance Manual" has become the standard for assessing dredge materials proposed for ocean disposal (U.S. EPA/ACE, 1991), dredge materials proposed for inland disposal (U.S. EPA/ACE, 1993 draft), and several scientific studies. Fortunately, the procedures in this document appear to have stood the test of time relatively well. Nonetheless, there are several modifications. These modifications, as well as procedures for freshwater organisms, are being incorporated into an ASTM guidance document that should be out in 1994 or 1995.

I. VERSIONS OF GUIDANCE MANUAL

The 1989 version of the Guidance Manual was not published and was distributed only as photocopies. In the 1989 version, Table IV-3A in the Appendix IV was in error. In 1991, the Army Corps of Engineers agreed to photocopy and distribute the Manual in support of the new "Green Book" (U.S. EPA/ACE, 1991). This version was single spaced and contained a corrected Table IV-3A. The 1993 version contains the corrected Table IV-3A, corrects an error in Figure IV-1, and makes a few editorial corrections.

II. NON-INDIGENOUS TEST SPECIES

Over the last few years, there has been a growing awareness of the ecological and economic damage caused by introduced species. Because both east and west coast species are often used in bioaccumulation tests, there is a real potential of introducing bioaccumulation test species or associated fauna and flora (e.g., pathogens, algae used in transporting the worms). Any user of this document needs to understand that it is their responsibility to assure that non-indigenous species are not released into the environment.

The general procedure to contain non-indigenous species is to collect and then poison all water, sediment, organisms, and associated packing materials (e.g., algae, sediment) before disposal. Chlorine bleach can be used as a poison. Double containment system is used to keep any spillage from going down the drain. Guidance on procedures used in toxicity tests can be found in Appendix B of DeWitt et al. (1992). Permits to import, hold, and use non-indigenous species may be required by state Fish and Game departments or other state agencies. Flow-through tests can generate large quantities of water, and the researcher needs to plan on having sufficient storage facilities.

III. SEDIMENT RENEWAL

We no longer recommend the addition of supplemental sediment

during 28-day exposures (Chapter X). In tests longer than 28 days, sediment addition is still an option to maintain pollutant concentrations and food quality/quantity as is periodic renewal of all the test sediment.

IV. DURATION OF TEST

Our recent work on field sediments contaminated with DDT, DDT metabolites, and dieldrin indicates that 28 days may be insufficient to obtain 80% of steady-state tissue residues for a number of higher K_{ow} compounds (see Boese and Lee, 1992 for summary of unpublished data and an expanded review of the percent of steady-state obtained in 28 days). These results emphasize the importance of conducting tests of at least 28 days duration.

In tests where it is critical to measure the residue within $\geq$80% of steady-state, it may be necessary to conduct longer term tests or at least to expose a few individuals for a longer duration to test whether steady-state was achieved. In screening tests where is important not to underestimate the residues, the residues obtained from 28-day tests could be multiplied by a "steady-state correction factor". The "steady-state correction factor" is the reciprocal of the decimal fraction of the amount of steady-state tissue residue obtained after 28 days. For example, if the tissue residue after 28 days is 0.33 of the residue at steady state, the correction factor would be 1/.33 = 3. These correction values would be obtained from previously conducted lab studies (e.g., Table IV-1 of Guidance Manual; Boese and Lee, 1992).

V. GUT PURGING

As far as we are aware, the effects of gut purging have not yet been assessed quantitatively. Therefore, the recommendations in Chapter X and Appendix X-1 remain the best available guidance.

VI. TERMINOLOGY AND COMPARISON OF BIOACCUMULATION MODELS

Unfortunately, the terminology used in bioaccumulation is not standardized and has continued to evolve, or at least change, since the Guidance Manual was written. The "thermodynamic-based bioaccumulation" model referred to in Appendix I-1 of the Guidance Manual is better termed the "equilibrium partitioning bioaccumulation" (EqP) bioaccumulation model. Since the term "accumulation factor" (AF) of the EqP bioaccumulation model first appeared in the peer-reviewed literature, the term "biota sediment accumulation factor" (BSAF) has been used by some authors. It appears that the two terms are interchangeable, though the reader is cautioned to check the units. Though it was not emphasized in Appendix I-1 of the Guidance Manual, AFs have units of gC/gL (see Lee, 1992) and it is critical that both the tissue pollutant and lipid concentrations be measured in or converted to the same units

(i.e., both wet or both dry). Additionally, the sediment pollutant concentrations and the total organic carbon (TOC) concentration of the sediment need to be in consistent units.

The guidance manual for open water disposal of dredge materials (U.S. EPA/ACE, 1991) uses the "theoretical bioaccumulation potential" (TBP). The TBP is the application of the EqP model using an accumulation factor of 4.0. Again the reader is cautioned to use consistent units for lipid and tissue residue and for sediment and lipid concentrations.

We now recommend the use of "k_s" rather than "k_1" for the first-order uptake rate coefficient from sediment to help distinguish it from uptake from water (see Lee, 1991). It is also recommended to consider k_s a coefficient rather than a constant.

More thorough comparisons of bioaccumulation models than given in Appendix I-1 can be found in Boese and Lee (1992), Landrum et al. (1992), and Lee (1992).

VII. FIELD VALIDATION

The bioaccumulation test methodology is presently being evaluated by comparing residues in field-captured Macoma nasuta with residues in laboratory-exposed M. nasuta and Nereis virens. The initial data for sum DDT with M. nasuta indicates that only 34% of the steady-state residue was obtained within 28 days at the one station where a long-term (90 day) test was conducted. However, when the 28-day residues from other stations were multiplied by the steady-state correction factor, the lab residues were within 3-fold of residues in field-captured M. nasuta in 7 of 8 cases. This comparison included 6 sites that varied by almost 3 orders-of-magnitude in sum DDT sediment concentrations, indicating that the procedures work over a wide range of sediment contamination.

VIII. REFERENCES

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U.S. EPA/ACE. 1993. Evaluation of Dredged Material Proposed for Discharge in Inland and Near Coastal Waters - Testing Manual (Draft).

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TABLE OF CONTENTS

APPENDICES
(Numbered sequentially by Chapter)

LIST OF TABLES

(Numbered sequentially by Chapter or Appendix)

TABLES IN APPENDICES

LIST OF FIGURES

(Numbered sequentially by Chapter or Appendix)

FIGURES IN APPENDICES

ABSTRACT

GUIDANCE MANUAL: BEDDED SEDIMENT BIOACCUMULATION TESTS

Henry Lee II, Bruce L. Boese, Judy Pelletier, Martha Winsor, David T. Specht, and Robert C. Randall

Bioaccumulation tests with bedded sediments are the most direct method of deriving tissue residue data required for evaluation of dredge materials and for quantitative ecological and human risk assessments. Bioaccumulation tests are also an important experimental tool for identifying the factors regulating the bioavailability of sediment-associated pollutants and to test various Sediment Quality Criteria approaches. However, the procedures for conducting such tests have not been standardized, making it difficult to compare studies. This manual gives detailed guidance on how to conduct "routine" bedded sediment bioaccumulation tests with marine or estuarine deposit-feeding organisms. All phases of the process are covered, from formation of the experimental design, through the actual exposures, to statistical analysis and interpretation of the results.

Because the interpretation of tissue residue data is often relative to "control" and "reference" sites, the acceptability of such sites is considered. The importance of an appropriate experimental design, including sufficient statistical power and replication, is stressed. Based on recommendations for statistical power and the use of one-tailed tests, a minimum of eight replicates is recommended. Methods to avoid or reduce "pseudoreplication, a common statistical problem in toxicity tests, are also discussed.

For the data generated from the bioaccumulation tests to be useful in quantitative risk assessments, tissue residues should be within 80% of the steady-state tissue residue. Based on a review of both 10-day and 28-day tests, we conclude that the 10-day test will not meet this criterion for many environmentally important pollutants, such as PCBs and DDT. Therefore, we recommend a 28-day exposure as the routine duration for bioaccumulation tests. Techniques for conducting long-term exposures (>28 days) and kinetic approaches based on uptake and depuration rates are also presented for cases when more accurate estimates of steady-state tissue residues are required.

Sediment collection and preparation, including spiking techniques, are discussed as are techniques for collecting and maintaining test species in the laboratory. Based on a number of criteria, including a required criterion for sediment-ingestion, five species are recommended as suitable for routine testing.

Another eight species are identified as potential "secondary" species. The water quality and sediment requirements for exposure chambers are discussed, and in most cases, these requirements can be achieved with relatively simple static or flow-through systems. Specific sampling schedules and techniques are given for the routine 28-day exposures. To allow comparisons among studies, we recommend the Bligh-Dyer method as the standard lipid technique, or, if another lipid method is used, to intercompare with Bligh-Dyer.

The statistical analysis of the data is discussed, and the use of one-tailed tests is recommended when comparing a test tissue residue(s) to reference or control tissue residue(s), as would commonly be the case when testing for "no further degradation". Besides the "no further degradation approach", other regulatory strategies for using tissue residue data are presented.

CHAPTER I. INTRODUCTION

Sediments are the ultimate sink for many of the pollutants entering the marine/estuarine environment through industrial and municipal discharges, dredge materials, and non-point runoffs. Sediments are an especially important repository for compounds that sorb strongly to particles, such as organic pollutants with high octanol-water partitioning coefficients (Kow) (e.g., PCBs, DDT) and many of the heavy metals. As a general rule, these are the same compounds that bioaccumulate to high levels. Accumulation of pollutants in bedded sediments (i.e., deposited rather than suspended sediments) reduces their direct bioavailability to pelagic organisms but increases their exposure to benthic organisms.

Bioaccumulation of these sediment-associated pollutants by benthic organisms can result in a number of ecological and human health impacts. Bioaccumulation of pollutants can result in acute and chronic effects in individual benthic organisms, which ultimately can translate into alterations in benthic community structure and function. Because the benthos are the primary food for demersal predators, predation on contaminated benthos is an important pollutant uptake route for many ecologically and economically important fishes and invertebrates. Once introduced into the pelagic foodweb, trophic transfer can spread the pollutants to higher trophic levels, including sea birds, marine mammals, and human consumers. If tissue concentrations in

shellfish or fishes exceed state or Federal regulatory criteria, such as the FDA Action Levels, areas may be closed to commercial and recreational fishing with the resultant economic loss.

Given the importance of bioaccumulation by benthic organisms, regulatory agencies need scientifically credible, cost-effective methods to measure benthic tissue residues resulting from exposure to existing sediments, as well as methods to predict tissue residues resulting from projected levels of sediment contamination. Additionally, development of standardized protocols to assess the bioavailability of sediment-associated pollutants are required to assist in the development of Sediment Quality Criteria. This need to assess or predict tissue residues in benthic organisms has long been recognized, and a variety of field, laboratory, and theoretical approaches have been developed, as discussed in Appendix I-1.

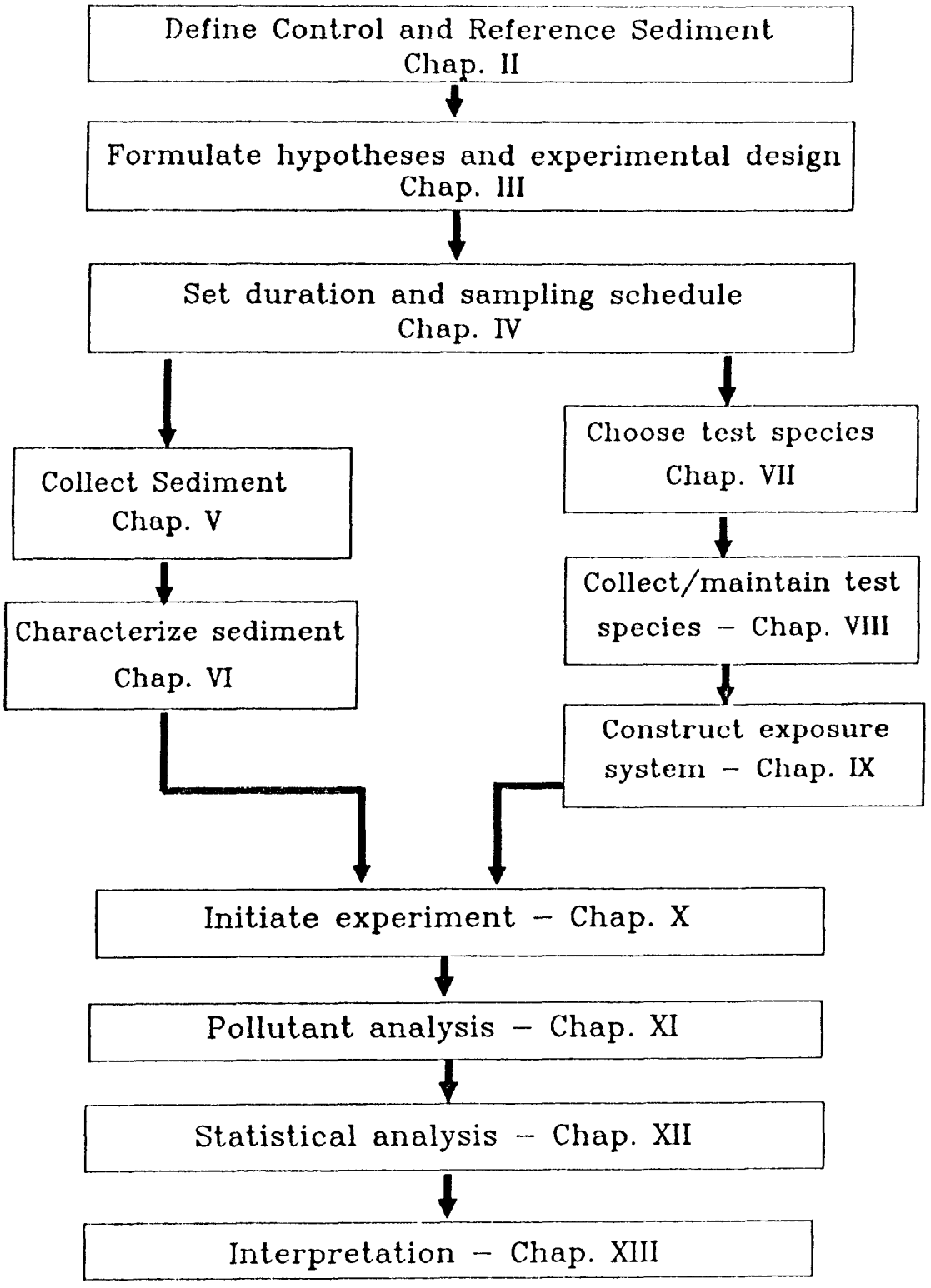
Of these approaches, the laboratory test offers great promise both in assessing existing field sediments or dredge materials and as an experimental technique to gain insights into the factors regulating bioavailability. Unfortunately, the techniques for conducting sediment bioaccumulation bioassays have varied considerably depending on the specific regulatory or experimental goals, making it difficult to compare results. Probably the most frequently used procedure has been the 10-day test to assess "bioaccumulation potential" of dredge materials (U.S.EPA/U.S.ACE, 1977), though recent evidence indicates the 10-day exposure is not adequate (see Chapter IV and Appendix IV-1).

The purpose of this manual is to develop a standardized approach to conducting sediment bioaccumulation tests with sediment-ingesting organisms exposed to bedded marine/estuarine sediment. The manual covers all aspects of the bioassay procedure, from experimental design to interpretation of the results (see Figure I-1), though detailed guidance in certain areas (e.g., analytical methods) is beyond the scope of this manual. These guidelines are designed for the "routine" testing of sediments and are not tailored toward any specific regulation or geographical location. The data obtained from the recommended 28-day test should, in most cases, generate the type of information required for quantitative ecological and human health risk assessments. Users of this manual must recognize, however, that the proposed standard 28-day test may underestimate actual steady-state field tissue residues under certain conditions. These possible sources of underestimation and alternate test methods are discussed.

The procedures presented here are based on our own experiences, results from numerous published sediment bioaccumulation tests, and to the extent appropriate, on the standard bioconcentration tests for water uptake (ASTM, 1984), the draft ASTM guidelines for sediment toxicity (ASTM, 1988a) and sediment storage, characterization, and manipulation (ASTM, 1988b), and the draft Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters (U.S. EPA/U.S. ACE, 1988) in preparation by Battelle. The reader is cautioned that the

FIGURE I–1

Steps in Conducting Sediment Bioaccumulation Tests



final versions of these draft documents may differ in their recommendations, and the final documents should be consulted.

The reader is also cautioned that the area of sediment bioavailability/bioassays is highly dynamic and there are diverse opinions among these various sources. When there is no consensus, our rationale for recommending a technique is discussed in the text or one of the appendices. A manual such as this must be considered a "living" document, as modifications will inevitably be needed both as a result of future research and as we gain a better understanding of the information required for quantitative risk assessments.

Though the methods in the manual have been directed toward "routine" 28-day tests with marine/estuarine sediment, many of the laboratory procedures are applicable to experimental exposures of different durations and to brackish/freshwater sediments. The standard 28-day test recommended here is not exclusive of other methods to assess or predict bioaccumulation (see Appendix I-1). The specific technique(s) used will depend upon the accuracy and precision required and the specific goals. In many cases, the various approaches complement each other and could be used sequentially or concurrently.

When working with contaminated field sediments or experimentally spiked sediments, adherence to safe laboratory practices is critical at all stages, as is disposal of all wastes in an environmentally proper and legal manner. The specifics of laboratory safety and waste disposal are beyond the scope of this manual, but some guidelines are given in Appendix I-2.

CHAPTER II: CONTROL VERSUS REFERENCE SEDIMENT

Tissue residues occurring in organisms exposed to the test sediment(s) are statistically compared to those occurring in organisms exposed either to "control" or "reference" sediments. Thus, the difference between control and reference sediments is critical to the interpretation of the results. Before initiating a test, determine if a particular sediment constitutes an acceptable control and whether to use both a control and a reference sediment or just a control sediment.

A. DEFINITIONS OF CONTROL AND REFERENCE SEDIMENTS

A "control" sediment is a pristine sediment or, more practically, a sediment with very low levels of pollutants. Essentially any contaminants in control sediments originate from the global spread of pollutants and do not reflect any substantial input from local point or non-point sources. The comparison of the test sediment versus the control is a measure (within the statistical limits of the test) of any bioaccumulation from the test sediment beyond the inevitable global background contamination. The use of control sediment also provides information on any contamination from the seawater or the exposure system. To the extent possible, grain size, TOC, and other key physical characteristics of the control sediment should closely resemble those of the test sediment.

In comparison, a "reference" site may contain low to moderate levels of pollutants. There are two slightly different ways in which to use a reference sediment. In the first case, the reference sediment is used as an indicator of the localized sediment conditions exclusive of the specific pollutant input being studied. Such sediment would be collected near the site of concern, and would represent the background conditions resulting from any localized pollutant inputs as well as the global input. This is the manner in which reference sediment is used in the dredge material evaluations (U.S. EPA/U.S. ACE, 1988). This document states that reference sediment should be collected "near the disposal site but should not have been influenced by previous disposal of dredged materials." As the purpose is to compare the reference sediments to the dredge material (i.e., test sediment), the reference sediments should be similar to the dredge material in grain size, TOC, and other physical-chemical characteristics.

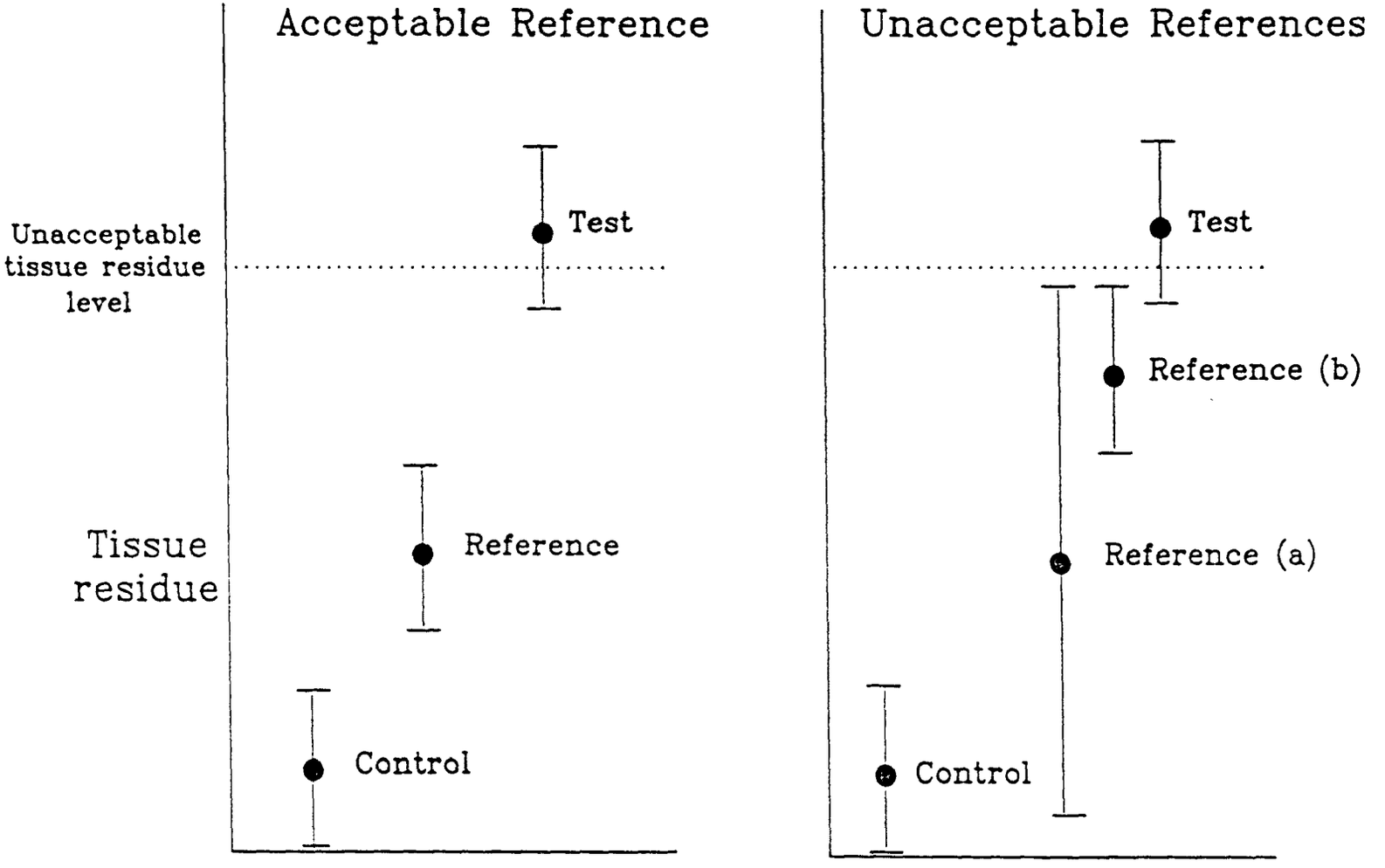
A second, though less common, use of reference sediments is as a measure of the tissue residues at a particular site before a specific pollutant input. This differs from the previous application in that the reference sediment is used as a measure of background conditions at the specific site rather than as an indicator of background conditions at another location. For example, if a sewage outfall pipe was being relocated in an urbanized near-shore environment, tissue residues in organisms exposed to sediment collected at the present discharge site (test sediment) would be compared to those in organisms exposed to

sediment from the new site (reference sediment). The difference in the tissue residues is an estimate of how much the relocation of the sewage discharge will increase the tissue residues at the new site. As the purpose of this comparison is to predict what will happen at a specific location, it may be impossible to closely match the physical-chemical characteristics of the reference and test sediments.

Understanding the type of information generated by reference sediments is critical for correct interpretation of the tests. Reference sediments usually contain measurable concentrations of a number of pollutants. Uncontaminated organisms exposed to reference sediments will eventually bioaccumulate pollutants to the level of the organisms living at the reference site. Comparing tissue residues from test sediments to those from a reference sediment determines whether the test sediment results in a significant incremental increase in tissue residues, not whether there is any bioaccumulation (i.e., bioaccumulation potential).

Use of a reference site is appropriate when a "no further degradation" approach is used to determine the suitability of an industrial or municipal discharge or a disposal operation (see Chapter XIII). Even in cases when the use of a reference sediment is appropriate, the sediment should not contain more than low to, at most, moderate levels of pollutants. If the reference sediment contains too high a level of a contaminant, the tissue residues in organisms exposed to reference sediment

FIGURE II–1
Overlap of Confidence Intervals in Organisms
Exposed to Acceptable and Unacceptable Reference Sediments



may not differ significantly from those in the test sediment even though the organisms exposed to the test sediments accumulated an unacceptable tissue residue (see Figure II-1). The situation in Figure II-1 is an extreme example, but it does illustrate that the results of a comparison of reference and test sediments depends upon the absolute level and the variation in the pollutant concentrations at the reference site.

B. CRITERIA FOR CONTROL AND REFERENCE SEDIMENTS

There are no simple criteria available to judge the acceptability of a sediment as a control or reference sediment. Ideally, the concentration of every anthropogenic pollutant (e.g., PCBs, DDT) in a control sediment should be significantly indistinguishable from zero, and the concentrations of naturally occurring compounds (e.g., metals) should be within natural levels. In practice, it will often be difficult to meet these criteria. One alternative is to use the pollutant levels in Table II-1, which gives a range of pollutant concentrations in control sites on the West coast, as a guide. Sediment with pollutant concentrations falling within the ranges in Table II-1 should represent adequate control values for the compounds measured. Alternatively, the concentrations at a putative control site can be compared to the sediment concentrations (normalized by the silt-clay fraction) given in NOAA (1988). This document presents raw data for both organics and metals for about 200 near-coastal sites throughout the United States with the concentrations for the highest and lowest 10 stations in

TABLE II-1: Representative Control Sediment Concentrations

COMPOUND	LOCATION		
	S. CALIF.[1]	PUGET SOUND[2]	YAQUINA BAY, OR [3]
BaP[a]	-	7-30	29-66
BF[b]	-	7-80	26.2
DDT	(15-150)*	0.03-0.6	<0.01
NAPH[c]	-	3-30[e]	<0.01[f]
PAH[d]	-	2-60	<0.01
PCB	(<5.0-18)*	<0.02-1.0	<0.01
Ag	0.06-2.0	1.2	0.55[g]
As	3-15	3-15	-
Cd	0.001-2	3.1-18.3	0.47
Cr	6.5-40	20.9	19.3
Cu	2.8-30	10-50	6.3
Hg	<1.0	0.02-0.12	-
Ni	<20.0	13.0	14.5
Pb	<10.0	8.0	5.5
Zn	<70.0	-	26.3

Organics are in ppb dry wt. Metals are in ppm dry wt.

* Not Considered Control Values

1 = Southern California (Bascom, 1984; Brown et al., 1984; Thompson et al.; 1984)
2 = Puget Sound, Washington (Konasewich et al., 1982)
3 = Yaquina Bay, Newport, OR (unpublished data)

[a]Benzo(a)pyrene
[b]Benzo(i,b,k)fluoranthene
[c]Napthalene
[d]Polyaromatic Hydrocarbons
[e](Brown et al., 1984)
[f](Schults, D.W., unpublished data. U.S.EPA, Mar. Sci. Ctr, Newport, OR)
[g](Swartz et al., 1984)

tabular form. Sediment concentrations falling within or near the lowest 10 stations would be acceptable as controls. Concentrations substantially above those in Table II-1 or the normalized values for the lowest 10 stations in NOAA (1988) should not be considered controls, with the possible exception of sediments containing natural high levels of certain metals.

Because the acceptability of a reference sediment depends in part on the local background pollutant levels and how the reference sediment will be used, no specific criteria will be suggested here. However, the appropriateness of the proposed reference site should be examined carefully if the silt-clay normalized concentrations fall in the upper half of the concentrations presented in NOAA (1988).

Because the regulatory interpretation of bioaccumulation tests is often based on a comparative approach, having scientifically defensible definitions and criteria for control and reference sediments are critical. The suggestions presented here represent a preliminary attempt at such criteria. To develop more rigorous criteria, a regional statistical analysis of the existing sediment data and any ecological effects data is required. Defining control and reference concentrations by their frequency of occurrence and by their correlation with adverse biological effects would then be possible.

It is important to emphasize that the comparison of a test sediment versus a reference sediment tests for an incremental

increase in bioaccumulation and not whether any bioaccumulation would result. Therefore, a control sediment treatment should always be used in addition to any reference sediments.

C. STANDARD REFERENCE SEDIMENTS

Variation in organism behavior and physiology can substantially affect pollutant uptake. For example, uptake in a test species could vary seasonally in response to changes in lipid content or temperature, or vary non-seasonally in response to organism health or site of collection. The extent of this variation should be assessed, especially if results will be compared from tests conducted at different seasons or from tests using organisms collected at different sites.

Organism variation can be assessed by using a "standard reference sediment," a well characterized sediment containing a known and constant pollutant concentration. This standard reference sediment treatment is a positive control and would be conducted in addition to the normal control, which is a negative control. Differences among studies in tissue residues in the standard reference sediment would measure the inherent variation associated with a test species. Use of a standard reference sediment would also help in standardizing results from different laboratories and/or different species.

Although positive controls have been suggested for sediment toxicity tests (e.g., Johns et al., 1989), they have not been adequately considered for use in sediment bioaccumulation tests. Part of the problem is the lack of any standardized sediment

suitable for bioaccumulation tests. In the absence of a national standard sediment, an interim solution is for each laboratory to make its own standardized sediment.

Because of potential spatial and temporal variations in pollutant concentrations in field sediments, the use of a laboratory spiked sediment is recommended as the standard. Spiking methods are discussed in Appendix V-1. The sediment used for the reference should be collected at the site where the test organisms are collected or, if that is impractical, the physical characteristics (e.g., grain size, TOC) should closely match those at the collection site. The unspiked sediment can be stored for long periods either by freezing or by drying. Although both of these processes can affect the physical integrity of a sediment, the purpose of the standard reference is to provide a constant exposure regime, not necessarily a natural one. Before either of these storage techniques are used, survival and/or behavioral bioassays should be conducted on previously frozen or dried uncontaminated sediment to assure that the technique does not adversely affect the test species. The sediment would be spiked just before its use.

Ideally, the standard reference sediment would be spiked with a suite of compounds ranging in chemical properties. Alternatively, a single neutral organic and/or a single metal could be chosen as a representative compound(s). A specific PCB congener, not an Aroclor, is a good candidate for the organics because of the wealth of information on PCBs, their high

bioaccumulation potential, and their resistance to metabolism. We suggest using 2,2',4,4',5,5' hexachlorobiphenyl (IUPAC #153), which is the most frequently occurring PCB congener in environmental samples (McFarland and Clarke, 1989) and is readily bioaccumulated by marine worms and clams (Rubinstein et al., 1987; McElroy and Means, 1988; Lake et al., in review; unpublished data). DDT is another possibility as an organic reference toxicant. The use of radiolabeled PCB or DDT is acceptable and would reduce the analytical load, though waste disposal of a mixed waste could be a problem. Cadmium is suggested as a general reference metal. Bioaccumulation of sediment-associated cadmium has been studied in a number of organisms (e.g., Ahsanullah et al., 1984) and has been suggested as the reference toxicant for Neanthes growth tests (Johns et al., 1989)

CHAPTER III. PRINCIPLES OF EXPERIMENTAL DESIGN

Care in experimental design is necessary to ensure the data generated are relevant to the problem under investigation, as well as to maximize the information gained relative to the cost. This chapter will discuss some basic principles of experimental design and provide examples as they relate to bioaccumulation tests. The following chapter presents specifics of the experimental design such as test duration and sampling schedule.

A. OBJECTIVES AND DEFINITIONS

The objectives of these experiments are to quantify the amount of pollutants bioaccumulated by organisms exposed to sediments or dredge materials and to test whether this accumulation is statistically greater than that occurring in a control or reference sediment. Each experiment consists of at least two treatments; the control and one or more test treatment(s). The test treatment(s) consist(s) of the contaminated or potentially contaminated sediment(s) or dredge material(s). A control sediment is always required to ensure there is no contamination from the experimental set-up, but some designs will also require a reference sediment (discussed in Chapter II). Uptake from the control sediment or from the reference sediment (when appropriate) is used to provide baseline values to compare with uptake from the test sediment. Thus, the reference sediment functions as the "control" treatment during comparisons with test sediment but also functions as a test

treatment during comparisons with the control sediment. Since the statistical term "control" treatment could be confused with the control sediment, in this chapter we will use the combined descriptor control/reference when referring to the sediment used as the "control" treatment.

The organism(s) to which a single application of treatment is applied is the experimental unit. This will be either a single organism or group of organisms (i.e., composite, see Section G) placed in an aliquot of a particular type of sediment in an exposure chamber. The specific type of sediment constitutes the treatment, for example, the dredge material is the test treatment. If a clam is placed in a beaker containing sediment, the clam is the experimental unit and the beaker is the exposure chamber. If several worms have to be composited to supply sufficient biomass for chemical analysis, the group of worms would constitute the experimental unit and the beaker or aquarium containing them would constitute the exposure chamber. If an aquarium is physically subdivided, such as containing several beakers each with an aliquot of sediment, then the organism(s) placed in each beaker is the experimental unit. The important concept is that the treatment (sediment) is applied to the experimental unit as a discrete unit.

Experimental units must be independent and not differ systematically. This chapter will discuss the procedures required to assure independence and randomization of the

experimental units, as well as the importance of replicating the experimental units to assure a sufficiently powerful statistical test.

B. HYPOTHESIS TESTING

Statistical testing requires the establishment of the null (Ho) and alternative (Ha) hypotheses prior to conducting an experiment. In most cases, the tests for the bioaccumulation bioassays will be one-tailed rather than two-tailed. One-tailed tests are used because the purpose of the experiment is to determine whether uptake from test sediment is significantly higher than uptake from control/reference sediment. If uptake is lower in the test sediment than in the control/reference sediment(s), presumably no testing is required (see Chapter XII). For these experiments, a one-tailed test will be performed where Ho is that the mean tissue residue of organisms in a test treatment is equal to the mean tissue residue of organisms in the control treatment. Ha is that the mean tissue residue of organisms in a test treatment is greater than the mean tissue residue of organisms in the control treatment. Each test treatment is compared to the control treatment separately.

Levels of statistical significance are stated by setting values for Type I and II errors. A Type I error occurs when Ho is rejected falsely (i.e., Ho is correct but rejected) and the probability of a Type I error is usually termed "alpha" and given a significance level of 0.05. In other words, if the tissue residues are equal (Ho is true) and the experiment were to be

repeated many times, an incorrect conclusion (i.e., Ho rejected with the conclusion that tissue residues are not equal) would occur 5% of the time. Type I error can be considered the "discharger's " risk as it is the probability of incorrectly ascribing bioaccumulation to a sediment or dredge material.

A Type II error occurs when Ho is falsely accepted (i.e., Ha is true) and is termed "beta". The converse of a Type II error (1-beta) is the statistical "power" of the test, which is the probability of correctly rejecting Ho (i.e., Ha is correct). We recommend a value of 0.05 for beta (power = 0.95) as the standard for the bioaccumulation tests. This means that if there were a true difference between test and control/reference tissue residues and the experiment were to be repeated many times, an incorrect conclusion (i.e., tissue residues equal when actually there is bioaccumulation) would occur 5% of the time due to chance. Type II error can be considered tne "environmental" risk as it is the probability of incorrectly concluding that a sediment or dredge material will not result in bioaccumulation. Using a one-tailed test, as recommended here, instead of a two-tailed test increases the power of the test given a set Type I error and reduces the number of replicates required.

By using the same value (0.05) for both Type I and Type II errors, an equal probability of error is assigned to both the "discharger" risk and the "environmental" risk. An implicit assumption of assigning equal risks is that the "cost" of making either type of error is equal. It is difficult, and often

subjective, to compare the monetary costs of pollution treatment/dredging to the environmental/human health costs of degradation of marine ecosystems. Therefore, in the absence of other data, we believe the use of equal risk is the most defensible procedure. In cases where it can be demonstrated that the "dischargers" risk is substantially greater than the "environmental" risk, the beta could be increased to 0.20, which would reduce the probability (power) of correctly detecting bioaccumulation to 80%.

Each pollutant must be considered and tested separately. Different pollutant tissue residues determined from the same experimental units are not independent and so can not be compared using the standard statistical tests. The appropriate statistical procedure for comparisons between different pollutants (e.g., comparisons of PCB congeners from the same tissue samples) is repeated measures ANOVA (see Chapter XII).

C. REPLICATION

An important principle in experimental design is the replication of experimental units. Replication is the assignment of a treatment to more than one experimental unit, which in the bioaccumulation experiment is the organism (or composite of organisms) to which a single treatment (e.g., test or control/reference sediment) is applied. The variation among replicates is a measure of the within-treatment variation which includes random variation among individuals as well as sampling

and analytical errors. This variation provides an estimate of within-treatment error used for assessing the significance of observed differences between treatments. In experiments without replication, inferential statistical testing is not possible.

A minimum number of replicates is needed for sufficient statistical power to determine whether the tissue residues of the test organisms are greater than those of the control. The number of replicates required can be calculated from an estimate of the variance or coefficient of variation of tissue residue values and a predefined minimum detectable difference between the two means. The minimum detectable difference is the smallest absolute difference between two means that is statistically distinguishable. For example, if the tissue residues in the organisms exposed to the test sediment must be at least twice as great as those in the control sediment to be statistically distinguishable, the minimum detectable difference of the means is 2.0. Besides the absolute difference between two means, the minimum detectable difference can be expressed as a proportion of the mean or as a proportion of the variance (see Appendix III-1).

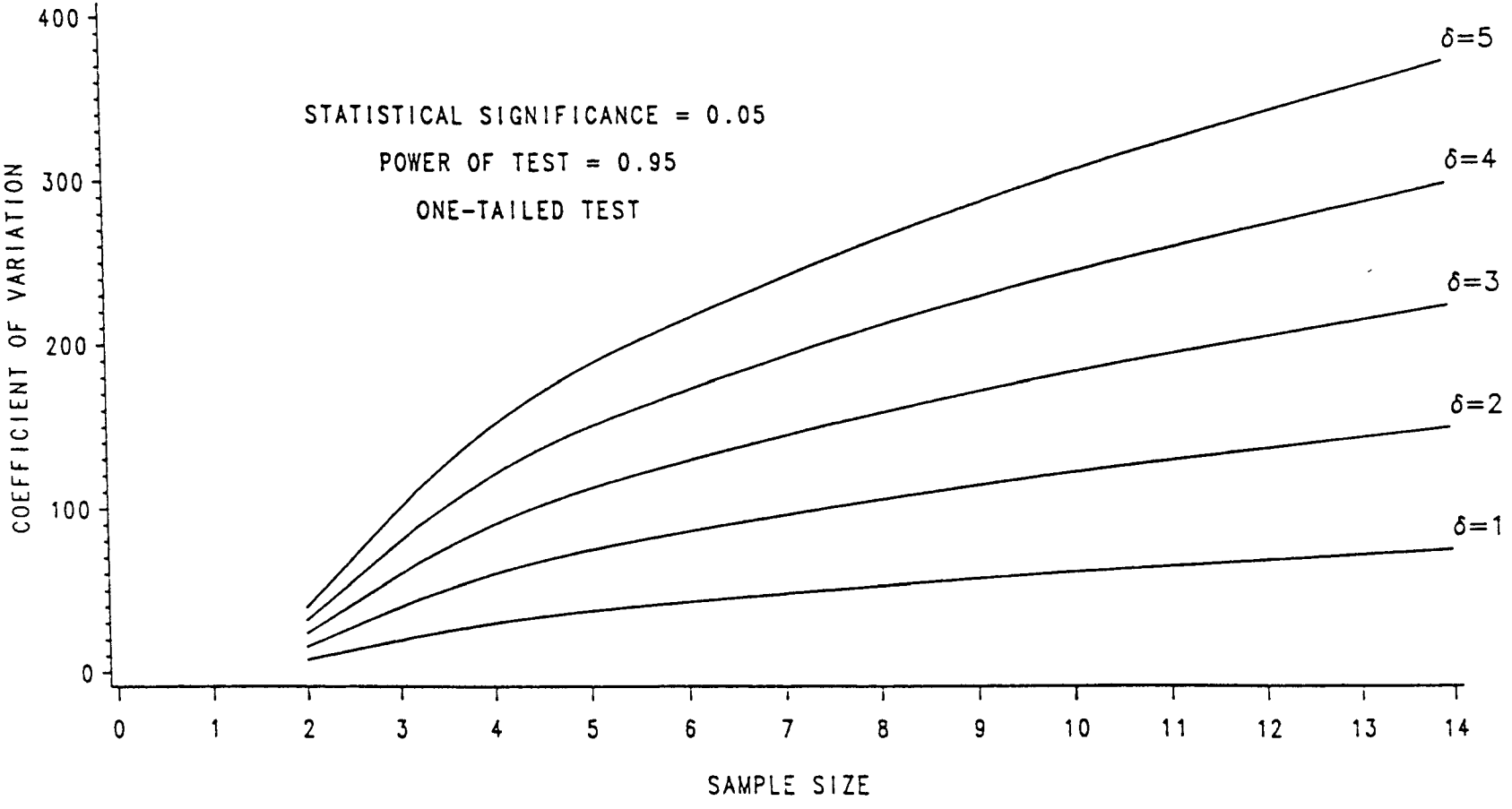
The smaller the minimum detectable difference, the greater the number of replicates required for a given significance level and power. Although there is no consensus on what constitutes an acceptable minimum difference, we suggest the bioaccumulation experiment be designed to detect a 2-fold difference between tissue residues in the test and control sediments or the test and reference sediments. In most cases, a 2-fold difference should

provide a sufficiently precise result to address the ecological and human health concerns. A smaller minimum detectable difference would be required if the 2-fold range around the control/reference sediment overlapped a human health or environmental criterion (e.g., FDA action limit), though this should be rare if the control/reference sediments are chosen correctly.

In some cases, it may not be necessary to distinguish a 2-fold difference in tissue residues among the treatments, such as when there are large differences between the treatments. For example, if the test sediment is suspected of being relatively contaminated (e.g. pollutant concentrations greater than 50 times control values), then a 5-fold minimum detectable difference would be sufficient to find significant differences between the treatments. In other cases, it may not be possible to achieve the 2-fold minimum detectable difference with a reasonable experimental design, such as with compounds with high analytical variation. When it is impractical to achieve the 2-fold minimum detectable difference, we recommend using sufficient replicates to distinguish a 5-fold difference in tissue residues between treatments, with the caveat that the 5-fold range around the control/reference does not overlap a sediment or tissue residue criterion or end-point.

Appendix III-1 provides instructions on computing the number of replicates (n). Figure III-1 may be used to determine n from a coefficient of variation and a minimum detectable difference

FIGURE III–1



COEFFICIENT OF VARIATION VS SAMPLE SIZE FOR VARIOUS MINIMUM DETECTABLE DIFFERENCES (δ)
EXPRESSED AS A PROPORTION OF THE MEAN

TABLE III-1: Ranges of Coefficient of Variations (CV) for Tissue Residues Reported for Benthic Organisms

POLLUTANT	ORGANISM	CV	REFERENCE
Cadmium	Modiolus demissus	4-54%*	1
	Mytilus edulis	4-61%*	1
	Mya arenaria	18-22%	2
	Mulinia lateralis	35-49%	2
	Callianassa australiensis	5-67%	3
Mercury	Modiolus demissus	5-34%*	1
	Mytilus edulis	5-53%*	1
Copper	Neanthes arenaceodentata	8-60%	4
Zinc	Nereis diversicolor	42%	5
	Octolasion tyrtaeum	12-30%*	6
	Corbicula fluminea	7-8%*	6
Kepone	Crassostrea virginica	8-80%	7
PCB	Octolasion tyrtaeum	2-23%*	6
	Corbicula fluminea	10-74%*	6,8
	Nereis virens	5-40%	9
	Uca spp.	31-75%	10
HCB	Macoma nasuta	23-33%	11
BaP	Amphipods	4-22%	12
	Macoma inquinata	4-36%	13
	Abarenicola pacifica	9-24%*	13
Napthalene	Macoma inquinata	50-100%*	14
Phenanthrene	Macoma inquinata	17-56%	13
	Abarenicola pacifica	10-31%*	13
Chrysene	Macoma inquinata	11-46%	13
	Abarenicola pacifica	2-46%*	13

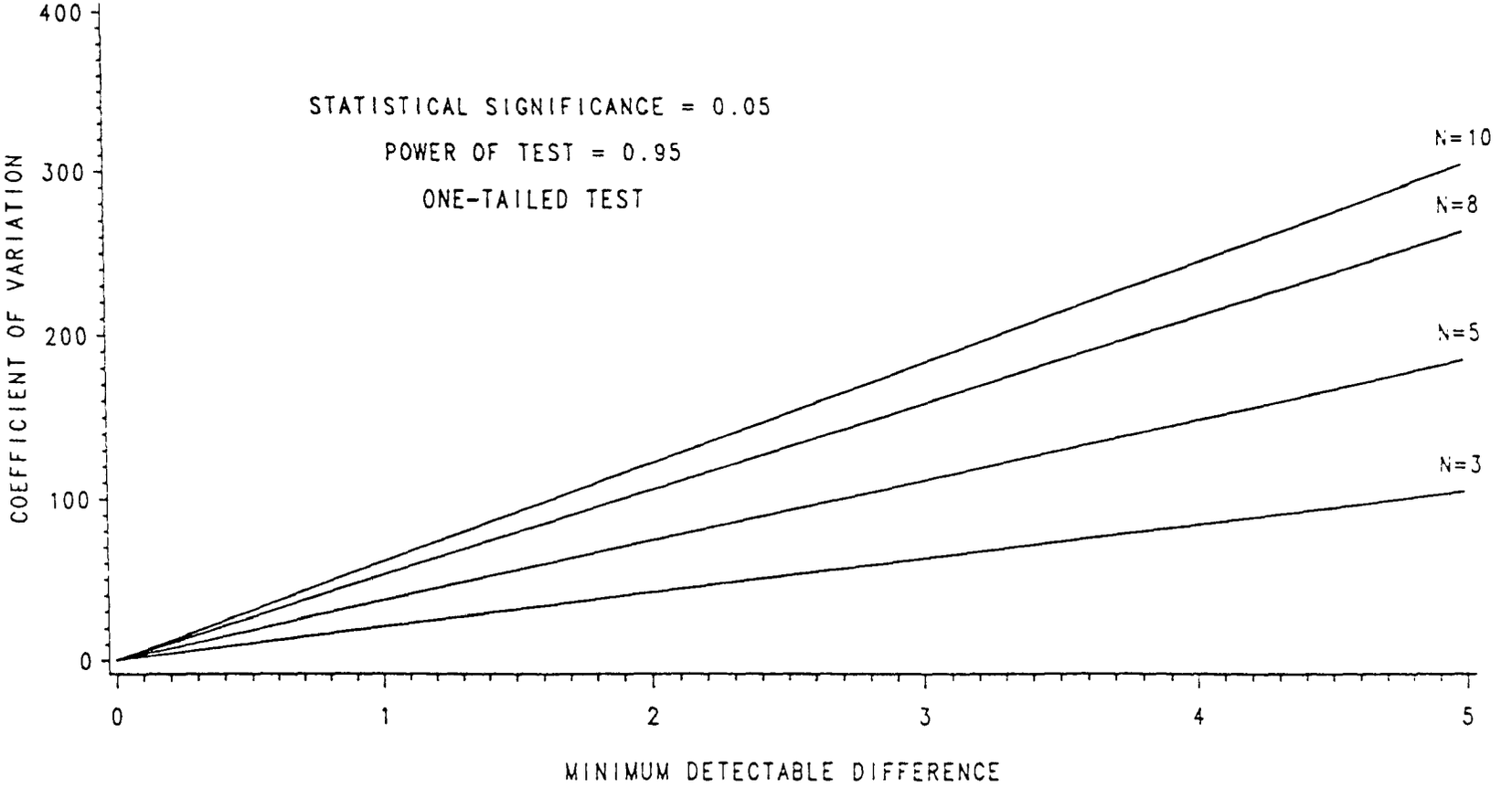
*Samples were composited resulting in (usually) lower CV's

1. Breteler and Saksa, 1985
2. Jackim et al., 1977
3. Ahsanullah et al., 1984
4. Pesch and Morgan, 1978
5. Renfro and Benayoun, 1975
6. Mac et al., 1984
7. Morales-Alamo and Haven, 1983
8. McFarland et al., 1985
9. Rubinstein et al., 1984
10. U.S. EPA, 1986b
11. unpublished data, 1989
12. Reichert et al., 1985
13. Augenfeld et al., 1982
14. Roesijadi et al., 1978

expressed as a proportion of the mean using a significance level of 0.05 and a power of 0.95 (beta value of 0.05) for one-tailed tests. If no other information is available, the coefficient of variations for tissue residues in various benthic species given in Table III-1 can used as guides for Figure III-1. The values in Table III-1 should be used as lower estimates as many were derived from composites (which will produce lower CVs than will individual samples), used radiolabeled compounds, and are the results from successful, published experiments.

ASTM (1984) recommends at least four replicates to determine bioconcentration factors. Because of the likelihood of a greater variation in sediment exposures compared to water exposures, we recommend a minimum of eight replicates as the "default" number of replicates to provide a statistical power of 95%. Figure III-2 may be used to determine if eight replicates are adequate for a specified coefficient of variation and minimum detectable difference expressed as a proportion of the mean. In some cases, when variability is low or less power is required, as few as five replicates can be used, though five should be an absolute minimum. In this discussion, the number of replicates refers to the number analyzed for tissue residues and not the number exposed. It is prudent to include an extra replicate or two for each treatment in case of mortality or the loss of samples during chemical analysis.

FIGURE III–2



THE MINIMUM DIFFERENCE DETECTABLE BETWEEN TREATMENTS
(EXPRESSED AS A PROPORTION OF THE MEAN)
FOR A SPECIFIC COEFFICIENT OF VARIATION AND SAMPLE SIZE (N)

D. RANDOMIZATION

Randomization is the unbiased assignment of treatments to the experimental units (i.e., organisms or composites of organisms) ensuring that no treatment is favored and that observations are independent. This is necessary for valid statistical testing. Randomization is often performed by using tables of random numbers. For these experiments, it is important to randomly assign the organisms to the control and test treatments, to randomize the allocation of sediment (e.g., not take all the sediment in the top of a jar for the control and the bottom for spiking), and to randomize the location of exposure units. For example, a bias in the results may occur if assignments are not randomized and all the largest animals are placed in the same treatment.

E. PSEUDOREPLICATION

The appropriate assignment of treatments to experimental units is critical to avoid a common error in design and analysis recently termed "pseudoreplication" (Hurlbert, 1984). Pseudoreplication occurs when inferential statistics are used to test for treatment effects even though the treatments are not replicated or the replicates are not statistically independent (Hurlbert, 1984).

1. Lack of Replication

The simplest form of pseudoreplication is treating subsamples as true replicates of the experimental unit (Figure III-3a). For example, two aquaria are prepared, one with

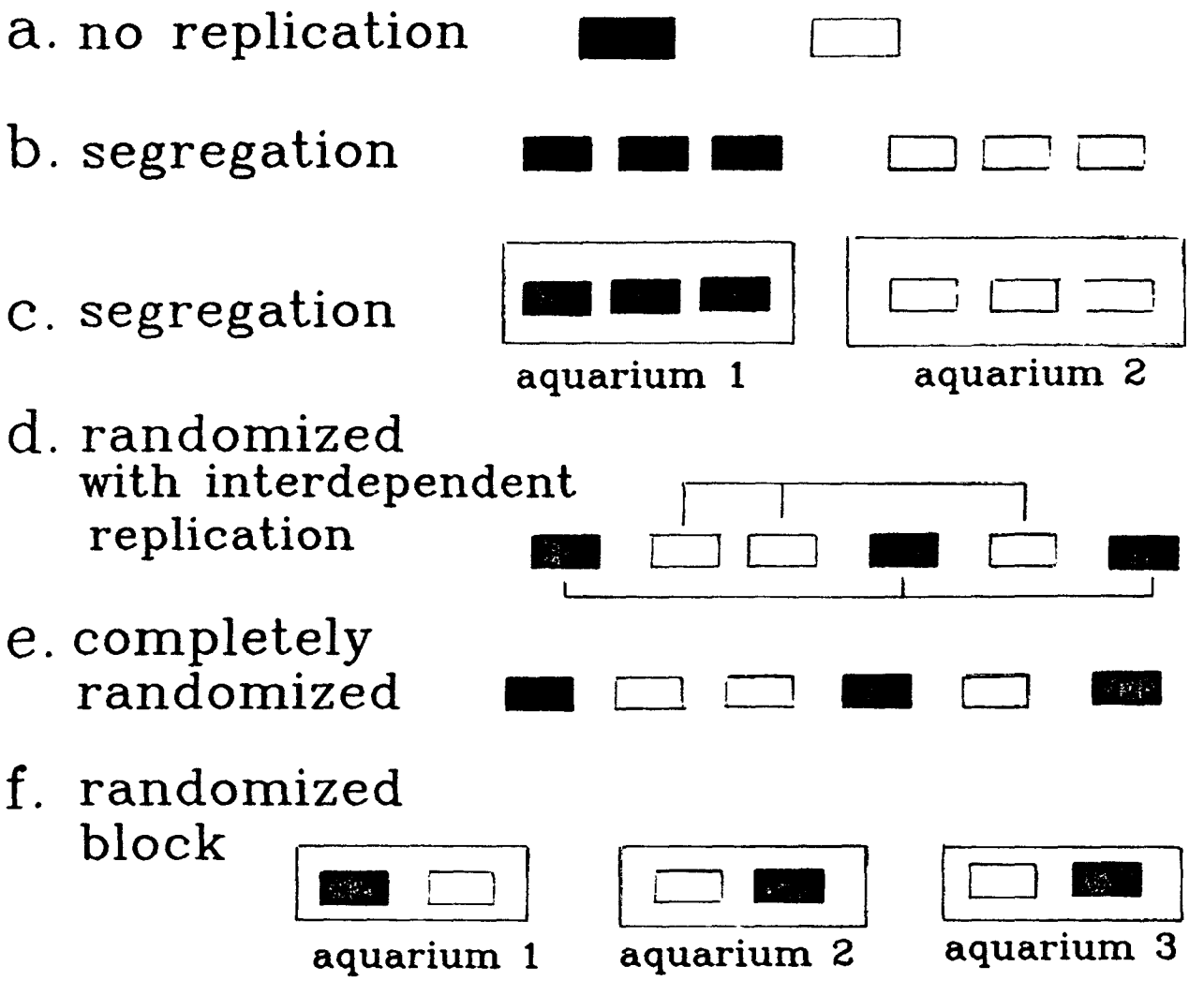
control sediment, the other with test sediment, and five organisms are placed in each aquarium. Even if each organism is analyzed individually, the five organisms are not true replicates because the treatment (i.e., sediment type) is applied to the aquarium as a whole and not to each individual organism separately. In this case, the experimental unit is the five organisms and each organism is a subsample, therefore, there is no replication of experimental units in this particular design.

2. Segregation

A less obvious form of pseudoreplication is the physical segregation of replicates by treatment, potentially resulting in a systematic error (bias) and lack of independence. For example, all the control experimental units are placed in one area of a room and all the test experimental units are in another (Figure III-3b). Spatial effects (e.g., different lighting, temperature) could bias the results for one set of treatments making it impossible to distinguish true effects of the treatment from the effects due to the physical layout of the experiment. Random physical intermixing of the experimental units is necessary to avoid this type of pseudoreplication.

A more common form of segregating replicates is the use of separate aquaria for each treatment. For example, segregation would occur if all the control experimental chambers (e.g., beakers) are placed in one aquarium and all the test experimental chambers in another aquarium (Figure III-3c). Any effects due to

FIGURE III–3
Random and Pseudorandom
Replication Schemes



control experimental unit

test experimental unit

a–d pseudoreplication

e–f strict replication

temperatures or different lighting conditions could bias the results for one of the treatments. Replicate aquaria are necessary in this case. Section III-F gives suggestions on addressing this type of pseudoreplication.

3. Randomization With Interdependent Replicates

Randomized spatial interspersion does not necessarily preclude pseudoreplication. If the replicates are physically interdependent, spurious effects can bias one treatment over another. This can occur if all the aquaria replicates of the control are serviced by the same water supply system while all the treatment aquaria replicates are serviced by another water supply system (Figure III-3d). Any differences between supply systems may potentially bias one set of aquaria over another. Thus, even if the aquaria replicates are physically interspersed, the replicates are not independent. To avoid pseudoreplication, each experimental unit should have its own water or air supply, all branching off a common supply and there should be no flow of water from one exposure system to another.

F. AVOIDING OR REDUCING PSEUDOREPLICATION

1. Avoiding Pseudoreplication

Pseudoreplication can be avoided by properly identifying the experimental unit, providing replicate experimental units for each treatment, and applying the treatments to each experimental unit in a manner that includes interspersion and independence. The simplest design that avoids pseudoreplication is the completely randomized design (Figure III-3e). In this design,

treatments are randomly assigned to the experimental units independent of location and each experimental unit is maintained in a separate exposure chamber with a separate water and air supply.

A randomized block design is also appropriate. A block is a set of relatively homogeneous units to which treatments are to be applied, such as all the beakers within an aquarium. In the randomized block design, all the treatments are randomly assigned to each block, and there are multiple blocks. For example, if there are two treatments and eight beakers per aquarium, each aquarium is randomly assigned four beakers with control sediment and four beakers with test sediment (for another example see Figure III-3f). One drawback of this design, however, is that since both test and control organisms are in one aquarium (block), the potential exists for contamination of controls by test sediment. This is especially likely with organisms that eject sediment into the water, such as Macoma during the production of pseudofeces. If this design is used, the aquaria and/or control exposure chambers need to be monitored to assure that cross-contamination does not occur.

2. Reducing Pseudoreplication Effects

Totally avoiding pseudoreplication may be difficult or impossible given resource constraints. For example, one common experimental design segregates the experimental treatments in separate aquaria. In this case, the beakers containing the test sediment are placed in separate aquaria from beakers containing

the control sediment (see Figure III-3C). Such a design avoids the problem of cross-contamination between the test and control sediment and does not require a separate aquarium for each beaker. However, because the beakers are segregated by treatment type, their distribution is not random. In such cases, the experimental unit may be defined as the replicated unit (organism(s) in the beaker with each beaker as a replicate), but with the stated assumption that there is no effect due to the physical segregation (aquaria effect in this example).

With this design, we recommend using replicate aquaria for each treatment type to enable comparison of results between aquaria within a given treatment using a nested ANOVA. If aquaria effects are apparent, the data from one or more aquaria may be considered invalid, or the differences due to the aquarium effect may be deemed trivial compared to the treatment effects. For example, if there is a significant difference among test aquaria results, but that difference is much less than the difference between test and control aquaria, the aquaria effects may be considered unimportant to the results of the experiment. However, moderate to large differences between aquaria of the same treatment would suggest a local contamination problem or other type of bias and the experiment should be repeated. If no significant aquaria effects are detected, the organism(s) within each beaker are properly considered the experimental unit and each beaker a replicate. The analysis (see Chapter XII) is then performed as if the beakers were not segregated into aquaria.

G. COMPOSITING SAMPLES

Compositing consists of combining samples (e.g., organisms, sediment) and chemically analyzing the mix rather than the individual samples. The chemical analysis of the mix provides an estimate of the average concentration of the individual samples making up the composite. Compositing will be used in bioaccumulation experiments primarily when the biomass of an individual organism is insufficient for chemical analysis. Several individuals can be composited into a single experimental unit with sufficient biomass and the analysis performed on the composite. Compositing is also used when the cost of analysis is high. For example, sediment pollutant analysis can be based on sediment composited from several exposure chambers of the same treatment to reduce the analytical sample load. Replicate sediment or tissue composites (i.e., experimental units) are required if statistical testing is planned.

For the tissue composite to be unbiased, the individuals must be randomly assigned to the various treatments. Each organism or sediment sample added to the composite must be of equal size (i.e., wet weight) and the composite must be completely homogenized before taking a sample. If compositing is performed in this manner, the value obtained from the analysis of the composite is the same as an average obtained from analyzing each individual sample (within any sampling and analytical errors). If replicate composites are made, the variance of the replicates will be less than the variance of the individual

samples, providing a more precise estimate of the mean value. This increases the power of a test between means of composites over a test between means of individuals or samples for a given number of samples analyzed.

However, if composites are made of individuals or samples varying in size (e.g., varying weights of sediment samples) or quality (e.g., disproportionate number of gravid females in one composite), the value of the composite and the mean of the individual organisms or sediment samples are no longer equivalent. The variance of the replicate composites will increase, decreasing the power of a test between means. In extreme cases, the variance of the composites can exceed the population variance (Tetra Tech, 1986a). Therefore, it is important to keep the individuals or sediment samples comprising the composite equivalent in size and quality. If sample sizes vary, consult the tables in Schaeffer and Janardan (1978) to determine if replicate composite variances will be higher than individual sample variances, which would make compositing inappropriate.

It is not advisable to composite samples if an estimate of the population variance is required or if information about the range in values obtained for individuals is needed. For example, tissue samples should not be composited if it is important to know the percent of individuals exceeding the FDA Action Limits. Compositing also requires more individuals (assuming individuals

can be analyzed) so it is not advised when space or cost keeps the number of individuals at a minimum. When there is extra sediment or tissue, archive individual samples in case a measure of the population variance or the concentration in a particular exposure chamber is desired latter.

CHAPTER IV: TEST DURATION AND SAMPLING SCHEDULES

Besides the statistical issues addressed in the previous chapter, an environmentally-relevant bioaccumulation test requires an appropriate exposure duration and sampling schedule for organism, sediment, and water samples. This chapter discusses these topics for the standard 28-day and long-term bioaccumulation tests, as well as a kinetic approach. Additional discussion of the 10-day versus 28-day tests are give in Appendix IV-1. Alternative test designs, which may be applicable under special circumstances or for research purposes, are presented in Appendix IV-2.

A. STANDARD 28-DAY BIOACCUMULATION TEST

1. Steady-State and Duration

Ideally, the duration of a bioaccumulation test should be sufficient for the organisms to reach steady-state tissue residues, where steady-state is operationally defined as the lack of any significant difference (ANOVA, alpha = 0.05) among tissue residues taken at three consecutive sampling intervals (ASTM, 1984). The time to reach or approach steady-state varies drastically among different compounds, but in general, the tests should be designed to generate environmentally-relevant data on high Kow organics (e.g., PCBs, DDT) and heavy metals. Therefore, we recommend a 28-day exposure as the standard duration. As discussed in Appendix IV-1, a 28-day exposure will result in tissue residues within 80% of the steady-state tissue residues in

most cases. When steady-state is not approached within 28 days, tissue residues of organics usually appear to be within 2-4 fold of steady-state concentrations (Table IV-1), which is considered acceptable for the ASTM bioconcentration test (ASTM, 1984). Although a 28-day sediment exposure will not assure attainment of steady-state for all environmentally important compounds, it will improve the predictive power of the test compared to the commonly used 10-day exposure. For cases where more accurate estimates of the steady-state tissue residues are needed, a long-term bioaccumulation test (Section B) or a kinetic uptake approach (Section C) is recommended.

2. Biotic Sampling Schedule

Biological samples are used to determine the amount of pollutants accumulated from the test sediment and to statistically compare these values to the amount of pollutants accumulated from control and reference sediments. To set the baseline conditions for these comparisons, bioassay organisms should be analyzed for pollutant and lipid content immediately before initiation of the experiment (t_0 samples). As discussed in the previous chapter (Chapter III), eight replicates are assumed as the number required to achieve sufficient statistical power. Therefore, eight replicate organisms or composites (i.e., experimental units) should be analyzed at t_0. The organisms sampled at t_0 should be chosen randomly from the same set of organisms used in the various sediment treatments. If compositing of individuals is necessary to obtain sufficient

TABLE IV-1: Percent of Steady-State Tissue Residue of Neutral Organics Obtained After 10 and 28 Day Exposures to Bedded Sediment.

Compound	% of Steady-State Tissue Residue 10-DAY	28-DAY	Species	Estimated by	Ref.
Phenanthrene	100	100	Macoma inquinata	G	1
Benzo(a)pyrene	96	100	Hexagenia limbata	K	3
Benzo(a)pyrene	96	100	Mysis relicta	K	3
Phenanthrene	94	100	Mysis relicta	K	3
Hexachlorobiphenyl	88	100	Hexagenia limbata	K	3
Phenanthrene	67	95	Pontoporeia hoyi	K	3
Aroclor 1260	53	100	Macoma balthica	G	4
Benzo(a)pyrene	43	75	Macoma inquinata	K	1
Chrysene	43	87	Macoma inquinata	G	1
Hexachlorobenzene	35	70	Macoma nasuta	K	2
Benzo(a)pyrene	32	66	Pontoporeia hoyi	K	3
Aroclor 1242	29	82	Cerastodema edule	G	4
Aroclor 1260	27	100	Cerastodema edule	G	4
Aroclor 1254	27	100	Cerastodema edule	G	4
Aroclor 1242	18	87	Nereis virens	G	4
Aroclor 1254	12	82	Macoma balthica	G	4
Aroclor 1254	9	25	Nereis virens	K	5

K = Steady-state tissue residue estimated from kinetic uptake model.

G = Steady-state tissue residues estimated from graphs of tissue residues versus time. Often, the 10-day and 28-day values had to be interpolated. "Steady-state" was defined as the time period with the maximum tissue residue.

SOURCES:
1 = Augenfeld et al., 1982
2 = Boese et al., in press and unpublished data
3 = Landrum and Poore, 1988
4 = Langston, 1978
5 = McLeese et al., 1980

biomass, the same compositing scheme should be used for all sampling periods throughout the experiment. At the end of the 28-day test period (t_{28}), eight replicate organisms or composites should be taken from each of the treatments and analyzed for pollutants and lipids. For comparing test and control sediments, the simplest design results in 24 tissue samples (8 controls at t_0, 8 controls at t_{28}, and 8 test at t_{28}).

We recommend including an extra one or two replicates in each treatment in case a sample is lost. Additionally, several extra individuals or composites should be taken at the initiation of the experiment. These extra samples should be frozen until the tissue residue data has been analyzed and interpreted. The method of physically sampling the organisms is discussed in Chapter X.

Time-series samples may be taken during the 28-day exposure to document uptake kinetics. This type of information can be very helpful even if it is necessary to limit the analytical load by taking only a single sample or, preferably, a single composite at each sampling period. However, if the data will be statistically compared to determine if steady-state has been attained, replicates are required at each sampling period. The sampling interval for these samples should approach a geometric progression with sampling periods of no greater than one week (e.g., day 0, 2, 4, 7, 14, 21, and 28). A sample at 10 days is recommended if there are previous 10-day exposure data. Begin the series on Monday to avoid weekend sampling.

3. Abiotic Sampling: Frequency and Replicates

The physical and chemical properties of each test, control, and reference sediment need to be characterized immediately after collection. This includes, at a minimum, grain size distribution, moisture content, pollutant concentrations, and TOC (or LOI) (see Chapter VI). Depending upon the length of storage, it may be necessary to remeasure these physical and chemical parameters, with the possible exception of grain size distribution, immediately prior to the start of the bioaccumulation test (i.e., t_0). If these t_0 samples will be statistically compared to samples taken at the end of the test period (t_{28}), eight replicate samples are required.

At the end of the bioaccumulation test (t_{28}), take sediment samples from each exposure chambers for measurement of pollutant concentrations, TOC, and moisture contents. It is usually not necessary to remeasure grain size. Preferably, these analyses should be conducted on the sediment from each beaker or aquarium (i.e., experimental unit). Measurements on individual experimental units may help explain any unexpected variation among the replicates. If eight replicates are used per treatment, this would result in a total of 24 sediment samples (8 controls at t_0, 8 controls at t_{28}, and 8 test samples at t_{28}).

If this is too large an analytical load, an alternative is to analyze a composite sample from each treatment composed of equal aliquots of sediment from each beaker or aquarium within the treatment. Additionally, a sediment sample from each beaker

or aquarium should be taken and archived. If the tissue residue data are more variable than expected or if there are "unusual" data points, these individual sediment samples should be analyzed. Additionally, individual sediment samples should be analyzed if the differences in pollutant concentrations in the t_0 and t_{28} sediment samples are greater than would be expected from analytical variation alone. It would then be possible to determine if significant changes in pollutant concentration or TOC had occurred during the course of the experiment.

B. LONG-TERM UPTAKE TESTS

1. Criteria and Limitations

In some cases, body burdens will not approach within 80-90% of the steady-state body burdens in a 28-day test (see Table IV-1 and Appendix IV-1). Organic compounds exhibiting these kinetics will likely have a log Kow > 5, be metabolically refractory (e.g., highly chlorinated PCBs, dioxins), and exhibit low depuration rates. Many of these same organic compounds biomagnify in aquatic food webs and pose a human health risk. Additionally, tissue residues of several heavy metals may gradually increase over time so that 28 days is inadequate to approach steady-state. Depending on the goals of the study, it may be necessary to conduct an exposure longer than 28 days (or a kinetic study as discussed in Section C) to obtain a sufficiently accurate estimate of steady-state tissue residues of these compounds. Although these longer term studies generate more accurate data for these compounds, they require greater resources, increase the

analytical load, and increase the likelihood of problems involving the maintenance of the organisms and temporal changes in sediment pollutant concentrations.

2. Biotic Sampling

In the long-term studies, the exposure should continue until steady-state body burdens are attained. As mentioned, steady-state is documented by the lack of any statistical difference in tissue residues in three consecutive sampling intervals (ASTM, 1984). ASTM (1984) recommends a minimum of five sampling periods (plus t_0) when conducting water exposures to generate bioconcentration factors (BCFs). For bioconcentration tests, ASTM (1984) recommends sampling in a geometric progression with sampling times reasonably close to S/16, S/8, S/4, S/2, and S, where S is the time to steady-state. This sampling design presupposes a fairly accurate estimate of time to steady-state, which is often not the case with sediment exposures.

To document steady-state from sediment exposures, we recommend placing a greater number of samples at and beyond the predicted time to steady-state. With a pollutant expected to reach steady-state within 28-50 days, samples should be taken at days 0, 7, 14, 21, 28, 42, 56, and 70. If the time to steady-state is much greater than 42 days, then additional sampling periods at 2 week intervals should be added (e.g., day 84). Slight deviations from this schedule (e.g., day 45 versus day 42) are not critical, though for comparative purposes, samples should be taken at t_{28}. An estimate of time to steady-state may be

obtained from the literature or approximated from structure-activity relationships (Appendix IV-3), though these values should considered the minimum times to steady-state.

Compared to the ASTM bioconcentration sampling schedule, this schedule increases the likelihood of statistically documenting that steady-state has been obtained though it does not document the initial uptake phase as well. If accurate estimates of the first-order uptake coefficient (k1, see Section C) are required, add sampling periods during the initial uptake phase (e.g., days 0, 2, 4, 7, 10, 14, 17, etc.).

One problem with longer exposures is the greater probability of the test organisms reproducing. Spawning can drastically affect lipid content and possibly pollutant concentrations (Niimi, 1983). Additionally, because many species die after spawning, it is prudent to add extra replicates. Increasing the total number of replicates by an additional 10-20% should suffice in most cases. If not needed, archive these extra individuals at the end of the test as replacement samples in case of analytical failures or analyze them to increase the statistical power of the final sampling period.

3. Abiotic Samples

The bioavailable fraction of the pollutants as well as the nutritional quality of the sediment are more prone to depletion in these extended tests than in the 28-day exposures. To statistically document whether such depletions have occurred, at least eight replicate sediment samples are required for physical

and chemical analysis from each sediment type at the beginning and the end of the exposure. Additionally, we recommend archiving sediment samples from every biological sampling period.

To minimize the depletion of sediment pollutants or nutrients, completely replace the sediment with stored sediment or freshly spiked sediment on a regular basis (e.g., monthly). Sediment must be renewed carefully to avoid damaging the test organisms, especially polychaete worms. Another way to minimize depletion of pollutants is to add fresh sediment periodically (see Chapter X). Over a long experiment, however, the exposure container may be entirely filled, necessitating the replacement of the sediment anyway. Replenishment sediment should be sampled and analyzed for the recommended parameters. Do not feed the organisms a supplemental food (e.g., fish flakes) as this will reduce exposure to ingested sediment and may result in an underestimation of sediment bioavailability and steady-state tissue residues.

C. ESTIMATING STEADY-STATE TISSUE RESIDUES FROM UPTAKE AND DEPURATION RATES

Several methods have been published which can be used to predict steady-state pollutant levels from uptake and depuration kinetics (Spacie and Hamelink, 1982; Davies and Dobbs, 1984). All of these methods were derived from fish exposures and most use a linear uptake, first-order depuration model which may be

modified for uptake of pollutants from sediment:

$$Ct(t) = k1*Cs/k2*(1-e^{-k2*t}) \quad (1)$$

Where: Ct = pollutant concentration in tissue at time t

Cs = pollutant concentration in sediment.

k1 = uptake constant (day^{-1})

k2 = depuration constant (day^{-1})

t = time (days)

As time approaches infinity, the maximum or equilibrium pollutant concentration within the organism (Ct_{max}) becomes:

$$Ct_{max} = Cs*k1/k2 \quad (2)$$

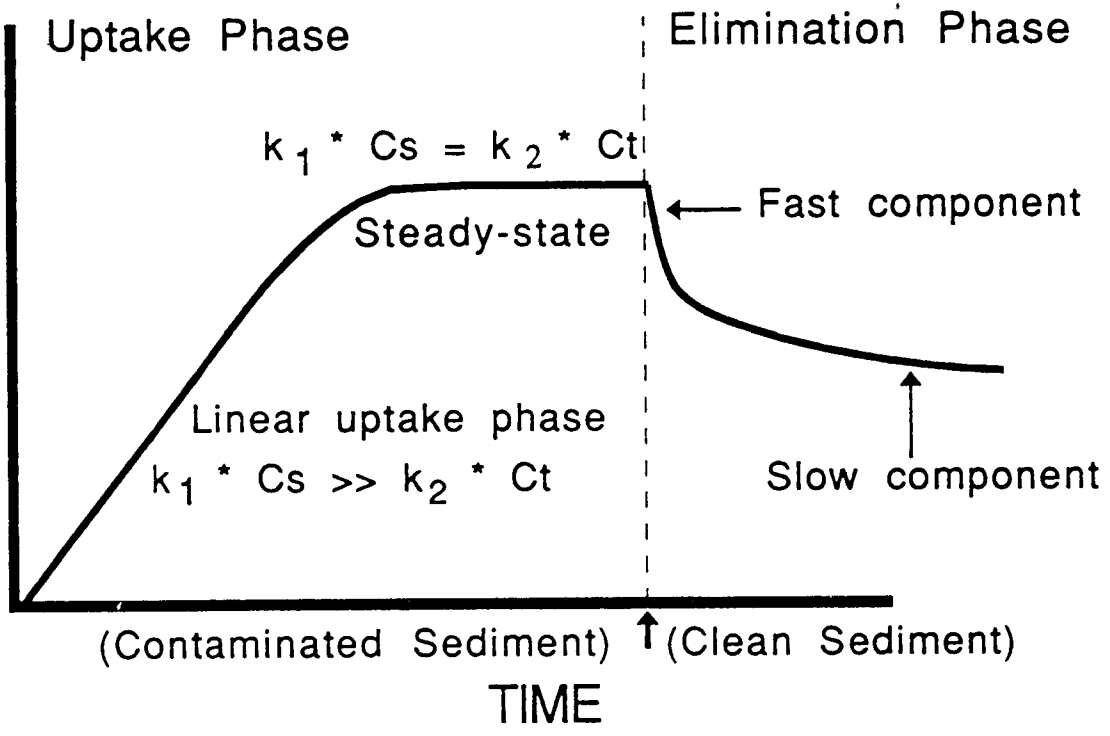
Correspondingly, the bioaccumulation factor (BAF) for a compound may be estimated from:

$$BAF = k1/k2 \quad (3)$$

The kinetic approach requires an estimate of the uptake rate constant (k1) and the depuration rate constant (k2), which are determined from the changes in tissue residues during the uptake phase and depuration phase, respectively. The uptake experiment should be short enough that an estimate of k1 is made during the linear portion of the uptake phase (Figure IV-1) to avoid an unrealistically low uptake rate due to depuration. The depuration phase should be of sufficient duration to smooth out any loss from a rapidly depurated compartment (Figure IV-1). Unless there is reason to suspect that the route of exposure will affect the depuration rate, it is acceptable to use a k2 derived from a water exposure. The durations of the uptake and depuration experiments will vary with animals species,

FIG. IV-1: Idealized Uptake - Elimination Curve

$$Ct(t) = Cs_1 * k_2/k * (1-e^{-k_2 * t})$$



Ct = tissue concentration (ug/g)
k_1 = uptake constant (1/time)
Cs = sediment concentration (ug/g)
k_2 = elimination constant (1/time)
t = time

compound, pollutant concentration, analytical detection limits, and test sediment. As a result, no specific guidelines will be presented here. For a discussion of this method for bioconcentration studies in fish, see Davies and Dobbs (1984), Spacie and Hamelink (1982), and the ASTM standard practice for conducting bioconcentration tests (ASTM, 1984). Effects of growth on the estimation of k1 and k2 and how to correct for growth dilution effects on the estimate of steady-state tissue residues are discussed in Appendix IV-2.

CHAPTER V. SEDIMENT COLLECTION, HOMOGENIZATION, MANIPULATION, AND STORAGE

Bioaccumulation tests use sediments collected in the field and brought back to the laboratory or manipulated experimentally in the laboratory. In both cases, the handling can result in loss of fine sediments, interstitial water, and water soluble compounds; oxidation of compounds; or contamination by metals and organics. This disruption can change physicochemical properties such as grain size distributions, pollutant concentrations, sorption equilibria, speciation, and complexation, thereby affecting pollutant bioavailability (Plumb, 1981; Jennett et al., 1980; Holme and McIntyre, 1984). Although some changes are unavoidable, they can be minimized with appropriate techniques. In this chapter, we provide guidelines on handling sediments during and after collection. We cannot, however, formulate a standard operating procedure applicable in all cases because the techniques used depend on the goal of the experiment and the pollutants of concern. In particular, techniques optimally suited to study metals may not be not be suitable for organic compounds (Plumb, 1981; ASTM, 1988b).

A. SEDIMENT COLLECTION AND TRANSPORT

1. Depth of Collection and Sediment Collection Techniques

The depths from which sediments are collected can affect bioaccumulation test results; therefore, a consistent depth should be used in all collections. We recommend sampling the upper 2-3 cm layer, a depth commonly used for toxicity and

bioaccumulation tests and sediment chemistry (e.g., Jenne et al., 1980; Plumb, 1981; Swartz et al., 1985a, 1986; NOAA, 1988; Ferraro et al., 1990). Advantages in sampling the upper layer include that the sediment is more recently deposited, more consistent in pollutant concentrations, in contact with the overlying water, and the most biologically active zone. However, tests on dredge materials may require that representative samples be collected from deeper layers, up to several meters deep, in areas intended for dredging.

To collect intertidal sediment samples by hand, use shovels, scoops, spatulas, or coring tubes. Hand skim or core with one of the above mentioned tools the upper 2 cm sediment layer. To maintain the sample layers intact, deposit the sediment sample into an appropriate container or, if a corer is used, plug the top and bottom of the tube. Core samples may be sectioned later at specific depth-intervals for analytical and bioaccumulation tests (Plumb, 1981; Holme and McIntyre, 1984; NOAA, 1988).

Box corers and benthic grabs are commonly used to collect sediments in subtidal waters. Sampler choice will vary according to firmness of substrate, volume of sediment needed, and type of ship available. Box corers are the preferred collection device because they disturb sediment layers the least and retain fine particles. A Smith-McIntyre or modified Van Veen grab, though more disruptive to sediment layers than a box corer, is acceptable. Compared to the box corer, these grabs operate in

sandier bottoms, are easier to handle, require fewer personnel, and operate in heavier seas (Plumb, 1981; Holme and McIntyre, 1984; Tetra Tech, 1986b; NOAA, 1988). Scrape surficial sediment from the grab or box corer samples and immediately store in appropriate containers. Consider flocculent material part of the sample (Lauenstein and Young, 1986).

If depth profiles are of interest, the original sediment layering must be preserved. Take core samples from the center of the grab sample once on shipboard and section them vertically at specific depth intervals (Plumb, 1981). To minimize oxidation and changes in other chemical properties, place plastic or Teflon^{R} bags or containers of appropriate composition and diameter over the ends of core tubes and extrude samples to specified depths.

Construct all collecting equipment with appropriate materials and clean equipment to reduce the possibility of contamination. When organic pollutants are the primary concern, avoid contaminating sediment with various plastics, especially if phthalate esters, used in flexible plastics, will be quantified. When metals are the primary concern, avoid contaminating sediments with any metal, including stainless steel. Subsampling from the center of grabs as well as coating, covering, or lining equipment with silicone rubber, Teflon^{R}, plastic, polypropylene, or polyethylene will eliminate direct contact of samples with the equipment. For instance, Teflon^{R} bags can be used to cover

stainless steel scoops (Lauenstein and Young, 1986). When both metal and organic contaminants are the interest, use glass when possible.

To remove organics/metal contamination, initially wash the equipment with a non-phosphate detergent, then consecutively rinse with distilled water, a water-miscible organic solvent, 5-10% hydrochloric or nitric acid, and finally deionized-distilled water (Plumb, 1981; Lauenstein and Young, 1986; U.S. EPA/U.S. ACE, 1988). Bake (>350^{o}C) all glass equipment (e.g., jars, corers, trays) before use. Glassware to be used in metal analyses should be stored wrapped in Teflon^{R} sheets or plastic wrap, whereas glassware to used in organic analyses should be stored wrapped in Teflon^{R} or aluminum foil. Wash new plastic containers and equipment as described and then leach them with distilled water before use. Rinsing grabs or corers with seawater between stations should suffice in most studies, though it may be necessary to use a brush or a detergent to remove highly cohesive sediments. When it is critical to remove all contaminants, Lauenstein and Young (1986) recommended rinsing grabs or corers with methylene chloride, followed by a seawater rinse. However, methylene chloride generates a hazardous substance, and its use on a ship would have to be carefully controlled so as not to endanger the workers or release a hazardous waste into the environment. Safer alternatives are methanol and ethanol alcohol.

Specifics of the field sampling design, such as the number of sites and the number of samples per site, depend upon the goals of the study and the type of spatial resolution required. Guidance for designing field sampling programs can be found in Green (1979), Elliott (1983), and Lauenstein and Young (1986).

2. Field Measurements

Upon collection, immediately determine sediment temperature and salinity by inserting a thermometer and an electrode 1 cm into the center of each sample (Plumb, 1981; Swartz et al., 1986). When metals are of interest, immediately measure pH and Eh, both of which require undisturbed samples (Holme and McIntyre, 1984). Important information recorded with each sample should include the site (name, with latitude and longitude to tenths of a minute), replicate number, depth, sampler description, numbers and kinds of subsamples, sediment characteristics, temperature, salinity, pH or Eh if measured, odor-color, penetration depth, sieve size, vessel size, date and time, weather conditions, names of chief scientist and team members, and comments (Lauenstein and Young, 1986).

3. Field Storage and Sediment Transport

Physical, chemical, and biological changes in sediment samples can occur rapidly, resulting in changes in sediment quality and/or bioavailability during the transport of sediment. Temperature, pH, and dissolved oxygen are often the rate controlling factors for these changes (Jennett et al., 1980). To diminish these effects, store the sediment sample in a bag or jar

immediately after collection. Teflon^{R} containers or brown borosilicate glass jars with Teflon^{R}-lined lids are recommended for both metal and organic samples, but regular glass jars with Teflon^{R}-lined lids are acceptable (Lauenstein and Young, 1986). Containers need to be completely cleaned and stored in a covered container to avoid contamination. Cleaning protocols used for the sampling equipment also apply to storage containers.

Fill jars and bags completely with sediment to eliminate airspace and retard oxidation of metals, but retain as much of the interstitial water as possible (Lauenstein and Young, 1986; U.S. EPA/U.S. ACE, 1988). Refrigerate sample containers in insulated cartons or ice chests immediately after collection. To maintain a temperature near 4^{O}C, provide containers with prefrozen, jelled refrigerant packs (e.g. Blue Ice^{R}) or ice. Make sure that samples are protected from the refrigerant to avoid cross contamination and freezing of the sample.

Shipping containers must be durable and leak-proof or lined with two heavy duty plastic bags. Add adequate absorbent material to soak up any leaked liquid. Pack samples tightly, using dividers between glass containers and fill all empty spaces with packing material. Mark containers with "This End Up" and "Fragile" labels. Ship samples by "overnight" or "24 Hour" carrier to the laboratory immediately after completion of sampling to protect sample quality. Refrigerate samples at 4C^{O} upon arrival. Guidance for shipping hazardous materials can be found in CFR 49, Parts 100-177.

B. SEDIMENT SPIKING AND MANIPULATION

Besides using field collected sediments, researchers can experimentally manipulate sediments to test pollutant bioavailability under defined conditions. Addition or spiking of pollutants to sediments is the most frequent type of manipulation. Other types of manipulations include the addition of inert substances for producing a less polluted sediment and the alteration of sediment organic content or particle size. Sediment spiking and manipulation techniques have not been standardized, and until standard methods are developed or the techniques are intercalibrated, exercise caution when comparing results from different techniques. Because manipulations can alter properties of sediments, prepare and manipulate control sediments in the same manner as test sediments. Several sediment manipulation techniques are outlined in Appendix V-1.

C. LABORATORY SEDIMENT STORAGE

Keep the time between the collection and/or spiking of a sediment and its use in bioassays to a minimum. If there is a delay of more than about 24 hours, store the sediment in air-tight containers at $4^{o}C$ in the dark. Because freezing may physically disrupt sediment, sediments for biological testing should not be frozen or freeze-dried (Tetra Tech, 1986b), with the possible exception of sediment stored for use as a "standard reference sediment" (Chapter II). If metals are the major source of contamination in a field sediment, the sediment should be

stored in the absence of air to minimize oxidation of reduced forms. Nitrogen can be used to fill the headspace in the container. Air-tight glass containers are recommended for sediments polluted with either metals or organics (Plumb, 1981; Lauenstein and Young, 1986). High density polyethylene and Teflon^{R} containers are also acceptable. Remove large organisms and extraneous material, such as bivalves or twigs, from the sediment before storing.

There is no consensus on maximum storage time other than that it should be kept to a minimum. This lack of consensus reflects the use of different end-points, sediment types, and storage procedures. Little information exists storage effects, though pollutants that are volatile, biodegrade rapidly, or undergo rapid oxidation-reduction reactions should be the most prone to changes in concentrations and/or bioavailability. Given the present state-of-the-art, the maximum time recommended for storage of dredge materials used for biological testing is 6 weeks at 4^{o}C (U.S. EPA/U.S. ACE, 1988), which seems reasonable. The storage of samples for analytical and physical analysis is discussed in Chapter XI.

D. SEDIMENT PREPARATION AND HOMOGENIZATION

Before using a field sediment, remove any extraneous materials (e.g., macroalgae, twigs, garbage, and rocks) and large organisms (e.g., bivalves). Disturb the sediment as little as possible during this process. The simplest technique is to

gently spread the material out in a glass pan and remove any large objects with forceps. However, if metals are of primary importance, keep contact with air to a minimum and use plastic tools.

If a field sediment is going to be experimentally manipulated (e.g., spiked with a pollutant), sieve the sediment through a 1-2 mm mesh sieve to remove the extraneous materials. Using as small a volume of water as possible, sieve the sediment over a large container (e.g., garbage pail). After letting the suspended fines settle for 6 to 24 hours, carefully siphon off or decant the overlying water and mix the settled fine particles back into the sediment.

After settling or storing sediments, mix them well immediately before taking aliquots for chemical analysis, spiking, or using in bioaccumulation tests. This will assure homogeneity as well as mix any separated interstitial water back into the sediment. Stir with a spoon or rod made of an appropriate material. If grab samples were divided into several containers, mix the respective sediment samples together before sampling or using in biological tests. Large masses of sediment can be manually mixed in an appropriately cleaned glass tray or plastic tub, or placed in jars and rotated on a rolling mill. Homogenize control sediments in the same manner as test sediments.

Visually inspect the sediment to judge the extent of homogeneity. Excess water on the surface of the sediment can

indicate separation of solid and liquid components. If a quantitative measure of homogeneity is required, take replicate (3-8) subsamples from the sediment batch and analyze for TOC, chemical concentrations, or the percent fines.

Jenne et al. (1980) cautioned against prolonged stirring which can abrade flocs and change the sediment's physicochemical properties, such as dissolved organic matter (DOM). However, all changes to the sediment are probably impossible to avoid. Recent results suggest that even stirring can increase interstitial water DOM concentrations (DeWitt, T. pers. comm. OSU, Mar. Sci. Ctr., Newport, OR).

CHAPTER VI. SEDIMENT CHARACTERIZATION

Bioavailability of sediment-associated pollutants depends on the physical and chemical composition of the sediment, which is often specific to a particular site and may change due to experimental manipulations (Gambrell et al., 1980; Jennett et al., 1980). Therefore, always measure the parameters potentially affecting bioavailability: pollutant concentrations, grain size distributions, organic carbon, and total solids contents (Plumb, 1981; NOAA, 1988). A number of other sediment and interstitial water measurements such as pH, Eh, cation exchange, and acid volatile sulfides will aid interpretation of the results, especially those of metals.

A. GRAIN SIZE ANALYSIS

Grain size analysis is the measure of the frequency and distribution of the disaggregated mineral particles comprising the sediment. Distributions are commonly reported on the Wentworth scale, which classifies particles as coarse sand, medium sand, fine sand, very fine sand, silt, and clay (Folk, 1980, Holme and McIntyre, 1984) (Table VI-1). Particle sizes are either expressed in millimeters or on a phi scale, where phi = $-\log_2$ particle diameter in millimeters. Quantification of the fine fraction (silt-clays <0.0625 mm or $\geq$ 4 phi) is important because pollutants predominantly associate with, and many deposit-feeders ingest, this fraction.

TABLE VI-1: Wentworth Grade Classification of Sediment

NAME	GRADE LIMITS	
	mm	Phi
Coarse sand	1.000 - 0.500	+1.0
Medium sand	0.500 - 0.250	+2.0
Fine sand	0.250 - 0.125	+3.0
Very fine sand	0.125 - 0.062	+4.0
Silt	0.062 - 0.004	+8.0
Clay	< 0.004	>8.0

The sand fraction of a sediment is determined by first drying the sediment (air dried or at <105^{o}C) and then removing the organic matter with 6% hydrogen peroxide (Buchanan, 1984). A known mass of dry sediment is then sieved through a standard series of sieves graded on the Wentworth scale (e.g., <.500, <.250, <.125, <.0625 mm, and pan) or on the phi scale (e.g., at 1/2 phi intervals) (Buchanan, 1984; Holme and McIntyre, 1984). Manually or mechanically shake about 25 grams of dry sediment for about 20 minutes and then weigh the contents of each sieve. To further characterize the silt-clay fraction (i.e, the sediment in the bottom pan), an hydrometer and pipette analysis is used (Jenne et al., 1980; Buchanan, 1984; Holme and McIntyre, 1984). Suspend the pan fraction in a graduated cylinder of deionized/distilled water, take periodic water samples from a known depth, and weigh the resultant suspended particles. The weight of the silt-clay fractions can then be calculated from the estimated settling velocities of particles of different sizes.

B. TOTAL SOLIDS CONTENT

Sediments are composed of both solids and interstitial water (IW), with the relative proportion of the two phases varying due to physical factors (e.g., percent sand) and biological factors (e.g., intensity of bioturbation). The total solids content, the percent of wet sediment comprised of particles, is used to convert sediment pollutant concentrations from wet to dry weights and to record changes in the ratio of water to sediment which can cause desorption of pollutants from sediment (Plumb, 1981).

Measure total solids content by drying a wet sediment sample at 105°C to a constant dry weight. The percent solids content is the dry weight divided by the initial wet weight multiplied by 100 (APHA, 1985). The percent moisture content of a sediment is 100% less the percent solids.

C. ORGANIC CARBON

Organic carbon content is one of the primary factors regulating sediment bioavailability (e.g., Rubinstein et al., 1983); therefore, measuring organic content on every sediment tested is critical. One direct measure of organic matter is total organic carbon (TOC), a measure of the total amount of oxidizable organic carbon. In comparison to TOC, total carbon (TC) is the measure of both the volatile organic and inorganic nonvolatile materials, such as carbonates and bicarbonates (U.S. EPA/U.S. ACE, 1988). Because of its relation to the binding of organic pollutants, TOC is the more biologically relevant measure.

Both TOC and TC can be determined by wet or dry (combustive) oxidation techniques (Plumb, 1981; Holme and McIntyre, 1984). Oxidation of an untreated (no acidification) sediment measures total carbon; whereas, oxidation of an acid treated sediment measures TOC (Plumb, 1981). The common wet methods use a chromic acid oxidation technique developed by Walkley and Black (1934; also see Holme and McIntyre, 1984) and el Wakell and Riley (1956). Buchanan and Longbottom (1970) describe a technique to

use on sediments containing coal. The differential combustion method, used in commercially available carbon analyzers, oxidizes the carbon with heat. The amount of CO_2 generated at different temperatures is a measure of the total and organic carbon. Carbon analyzers are simpler to use than wet techniques and are recommended. However, exercise care in using or purchasing carbon analyzers because instruments designed for measuring TOC in seawater may be too sensitive for sediments, requiring extensive dilution of the samples.

A commonly used method to estimate organic matter is loss on ignition (LOI) or total volatile solids (TVS), which is the percent loss of weight after combustion. The organic matter is combusted by heating dry sediment at 550^{o}C for 1-4 hours. LOI is calculated as the difference between the dried and combusted sample weights divided by the dry weight, multiplied by 100 to convert the number to a percent (Dean, 1974; Byers et al., 1978; Plumb, 1981; APHA, 1985). Structurally bound water and carbonates, found particularly in clays and in calcareous sands, may be lost along with volatile solids during combustion, distorting actual organic carbon values (Dean, 1974). Because LOI includes these non-organic constituents, it is not a direct measure of sediment organic carbon content.

When determining LOI, spread a small aliquot (a few grams) of sediment out thinly in a porcelain ignition dish at least 75 mm in diameter. This exposes the sediment to an adequate oxygen supply for oxidation of the organics. Clumps of sediment and/or

large amounts of sediment have a tendency to block oxygen transfer to the center of the clump, thereby yielding significantly lower and/or inconsistent LOI results. We analyze 2g samples of sediments and ignite the dry total solids residue for 1.5 hours at 550^{o}C.

Of the two methods, LOI is simpler and cheaper than TOC. However, most research and regulatory applications require a more accurate measure than LOI (see Dean, 1974; Byers et al., 1978; Mook and Hoskin, 1982). Because TOC is one of the parameters used to calculate Accumulation Factors (see Appendix I-1), we recommend using LOI as a rapid survey method, but performing TOC analyses on all sediments used in bioaccumulation tests. If analyzing TOC on all sediments is impossible, determine the conversion between LOI and TOC on a few samples or use the values in Table VI-2. However, use caution when using these conversion factors because the relationship between LOI and TOC can vary several fold among sediments (Dean, 1974; Byers et al., 1978; Ditsworth, G., pers. comm., U.S. EPA, Mar. Sci. Ctr., Newport, OR).

D. ADDITIONAL SEDIMENT CHARACTERISTICS

Measure salinity in the overlying and interstitial water during the initial characterization of sediments in the field. A refractometer measures salinity with sufficient accuracy in most cases and requires only a few drops of interstitial water. In studies of metal bioavailability, measure pH and Eh, both of

TABLE VI-2: Ratios For Converting Loss on Ignition (LOI) to Total Organic Carbon (TOC) For Various Sediment Types.

Particle size %>63um	Particle size %<63um	TOC	LOI	TOC/LOI	Reference
36	64	1	2.13	0.47	Dean, 1974
med. silt		10	22.9	0.44	Byers et al., 1978
63.5	36.0	1.21	4.30	0.28	unpubl. data
34.1	65.9	2.29	7.79	0.29	unpubl. data
50.2	49.8	2.10	6.53	0.30	Ditsworth[1], pers. comm. (Mean of 12 sediments)
19-81	19-81	0.53-3.50	2.43-10.10	.21-.37	Ditsworth[1], pers. comm. (Range of 12 sediments)

[1] G. Ditworth, U.S.EPA, Mar. Sci. Ctr., Newport, OR

which can affect pollutant solubility and mobilization (Engler, 1980). Measure these parameters during field collection, repeating the measurements after handling and storage during which values can change rapidly. Use a meter or probe, and a platinum electrode, to measure pH and Eh, respectively. Insert the probes 1-3 cm below the sediment surface and allow to stabilize (Plumb, 1981). Discussion of pH and Eh can be found in Fenchel (1969) and Pearson and Stanley (1979).

Additional characteristics potentially affecting metal availability are amorphic oxides of iron and manganese, iron sulfides, cation exchange sites, and selectively extractable fractions using extractants such as hydrogen peroxide, acetic acid, and organic chelates (Luoma and Jenne, 1976; Jennett et al., 1980; Plumb, 1981). Consider measuring these parameters in studies of metal bioavailability. Recent work suggests that acid volatile sulfides (AVS) may be the primary factor regulating the bioavailable fraction of many metals (DiToro et al., in review). If further investigations support the initial findings, it may be possible to use AVS as a "normalizer" for sediment metals much as TOC is used for neutral organics. Morse et al. (1987) gives a method to measure AVS.

E. INTERSTITIAL WATER

Interstitial water is an integral part of bedded sediments and pollutants associated with the interstitial water may play a major role in the uptake and toxicity of a number of compounds (Adams et al., 1980; Swartz et al., 1988 ; Landrum 1989).

Because of the potential role of pore water in controlling uptake and toxicity, its characterization can be very informative. "Dissolved" pollutant concentrations, complexed pollutant concentrations, and dissolved organic matter (DOM) are the most important parameters to measure.

A number of techniques have been used to collect interstitial water for pollutant or nutrient analysis, including squeezing of sediment cores (Presley et al., 1967; Robbins, 1977), centrifugation (Edmunds and Bath, 1976; Plumb, 1981; Landrum et al., 1984), suctioning (Plumb,1981; Knezovitch and Harrison, 1987), equilibration with dialysis membranes (Mayer, 1976) or porous Teflon^{R} cups (Zimmermann et al., 1978), and displacement with other liquids (Bately and Giles, 1980). Manheim (1974) published a comparative study and Bately and Giles (1980) reviewed several methods.

Centrifugation is a straight-forward technique suitable for the routine collection of small to moderate amounts of interstitial water for pollutant analysis. There is no standard procedure, but centrifuging the sediment sample at 7000 - 9000 rpm for 5 - 10 minutes should suffice in most cases (Bately and Giles, 1980; Plumb, 1981). Higher rpms are required if any suspended particles remain, because particulate matter in the supernatant will result in erroneously high "dissolved" pollutant concentrations. If the speciation of metals will be examined, the centrifuge tubes should be oxygen-free. Following centrifugation, the supernatant is often vacuum filtered to

remove particulate matter. There is no standard pore size, but a 0.45 um Nuclepore^{R} membrane filter is frequently used (U.S.EPA, 1979; Plumb, 1981; Landrum et al., 1984). The filters should be precleaned in 10% nitric acid for 24 hours and then soaked in deionized water for another 24 hours (Lapan, R. pers. comm. U.S.EPA, ERL-N, Narragansett, RI) A potential source of error is sorption of dissolved pollutants onto the filter, resulting in an underestimation of dissolved pollutant concentrations. Measure the sorption of the pollutants onto the filter by determining the loss of pollutants from standard solutions passed through the filter.

Pollutants in the interstitial water may either be truly dissolved or complexed with DOM (Engler, 1980). In general, the complexed pollutants have a lower bioavailability than the free forms (Luoma and Bryan, 1978; Jenne et al. 1980). As DOM levels may change due to handling (DeWitt, T., pers. comm., OSU, Mar. Sci. Ctr., Newport, OR) or biotic effects (e.g., excretion of organics), the bioavailability of interstitial water pollutants may change over the course of an experiment or vary between field and laboratory sediments. The concentration of DOM can be determined either by using wet oxidation methods (Walkley and Black, 1934; Holme and McIntyre, 1984) or a carbon analyzer. As mentioned, carbon analyzers that measure the relatively low concentrations of DOM in interstitial water with good accuracy are not as efficient at measuring the high concentrations of TOC in sediment.

Several techniques have been developed to measure the concentrations of the dissolved and complexed pollutants in interstitial water. A relatively simple technique has been developed by Landrum et al. (1984) to separate and quantify total, freely-dissolved, and colloidally-bound non-polar organic pollutants in interstitial water. This technique uses a Sep Pak C-18^{R} cartridge to separate the bound and dissolved fractions. The bound fraction of the pollutant will pass through, while the dissolved fraction will be retained by the column.

Besides direct measurement, equilibrium interstitial water pollutant concentrations can be calculated from the TOC, bulk sediment concentration, and Koc for a compound (Karickhoff, 1984). Because all the aforementioned collection techniques may alter interstitial water characteristics through adsorption/ desorption of pollutants or suspension of particulate matter (Word et al., 1987), the calculated value for interstitial equilibrium concentrations may be more accurate for very high Kow compounds (Karickhoff, S., pers. comm., U.S. EPA, ERL-A, Athens, GA). Calculated concentration values will not monitor temporal changes in interstitial water but will serve as check on directly measured concentrations. Measured concentrations substantially higher than calculated could indicate suspended particles; whereas, measured concentrations lower than expected could indicate either that equilibrium had not been attained or that the pollutant had adsorbed to the filter.

CHAPTER VII. ORGANISM SELECTION

A. SELECTION CRITERIA

The choice of the test species can have a major influence on the success, ecological significance, and interpretability of a bioaccumulation test. Given the potential range in environmental characteristics, no one species is best suited for all conditions. There are, however, two characteristics required of any bioaccumulation test species, as well as a number of other desirable characteristics. These characteristics are summarized in this chapter and Table VII-1 and discussed in more detail in Appendix VII-1.

The first required criterion is that the test species ingest sediment. This requirement is critical because recent work has demonstrated that ingested sediment is the major uptake route for higher Kow compounds for some species (Landrum, 1989; Boese et al., in press). Many benthic invertebrates are flexible in their feeding mode, and this requirement does not preclude the use of facultative filter-feeders (e.g., Macoma) as long as the only exposure route during the experiment is from bedded sediment (i.e, no resuspended particles or phytoplankton). The second required attribute is that the test species be sufficiently pollutant resistant to survive the duration of the exposure with a minimum level of mortality. This requirement precludes the species routinely used in sediment toxicity testing (e.g., Rhepoxynius).

Besides the required criteria, there are a number of desirable characteristics -- ease of collection, year-round availability, ability to culture the species, adaptability to laboratory conditions, suitable size, tolerance to a wide range of sediment types and salinities, suitability for sublethal or toxicokinetic tests, ecological or economic importance, having a high bioaccumulation potential, compatibility with other species, and a low capability of metabolizing PAHs and other contaminants. The importance of these various criteria depends upon the specific goals of the research and the sediment tested. However, using an organism large enough to supply sufficient biomass for chemical analysis is important in nearly all cases. Ideally, the test species should be large enough to allow chemical analysis on individuals. Even when individuals are composited, compositing a smaller number of larger organisms is easier than dozens or hundreds of smaller specimens.

B. RECOMMENDED SPECIES:

An evaluation of the suitability of potential test species is summarized in Table VII-1. This evaluation is not based on extensive comparative studies and should be considered a guide rather than a definitive characterization of the species. Based on this analysis, we identified five recommended bioaccumulation test species and another eight "secondary" taxa. The recommended species meet all or nearly all of the desired criteria and are well established as bioaccumulation test species. The recommended

TABLE VII-1

PERTINENT CHARACTERISITCS OF TEST SPECIES

SPECIES	Feeding Type	Biomass	S °/oo	Pollution Tolerance	Culture Potential	Commercial Availability	Bio. Info
Abarenicola spp.	Fun	++	>15	+	−	−	+
Arenicola spp.	Fun	++	>15	+	−	+	+
Callianassa spp.	SSDF	++	>10	−?	−	+	−
Capitella spp.	SDF	−	>10	++	+	+	++
Macoma balthica *	SDF	+	>10	+	−	−	++
Macoma nasuta *	SDF	++	>10	+	−	−	++
Nephtys incisa	SSDF	+	>25	+	−	−	+
Neanthes arenaceodentata *	SDF/O	+?	>28	+	++	+	++
Nereis virens *	SDF/O	++	>10	++	−	+	++
Nereis diversicolor *	SDF/O	++	>10	++	−	+	++
Nucula spp.	SSDF	+	?	+	−	−	+
Palaemonetes pugio	SDF	+?	>10	−?	+	+	++
Yoldia limatula *	SSDF	+	>25 ?	+	−	−	+

Fun = Funnel feeder
O = Omnivore
SDF = Surface Deposit Feeder
SSDF = Subsurface Deposit Feeder

\+ = good, sufficient
++ = very good
− = poor, insufficient

* Recommended test species

Bio Info. = information on bioaccumulation toxicity

species are the polychaetes Nereis diversicolor, Neanthes (Nereis) virens, and the bivalves Macoma nasuta, Macoma balthica, and Yoldia limatula. These species have been used in a substantial number of experimental bioaccumulation studies and in regulatory monitoring. Within their tolerance levels, these species should serve as suitable test species, and we recommend using at least one of these species in all tests, at least until the suitability of other species has been demonstrated locally.

The secondary bioaccumulation species meet the required characteristics but are to some extent deficient in one or more of the important desired characteristics and/or there is insufficient information to make a final evaluation. However, some of these secondary taxa offer potential advantages such as large size (arenicolid worms), additional phylogenetic groups (i.e., crustaceans), adaptability to culturing (e.g., Neanthes arenaceodentata), and high pollution tolerance (Capitella spp.). The importance of these various advantages depend upon the site specific situation (e.g., level of toxicity of sediment).

C. NUMBER OF SPECIES TESTED AND MULTIPLE SPECIES TESTS

Species as well as larger phylogenetic groups vary in their tendencies to bioaccumulate pollutants both in response to their modes of exposure and to their metabolic characteristics. The extent of these interspecific variations are not well understood, and both the magnitude and direction of species differences can vary with pollutant (e.g., metals vs. organics) and perhaps with

sediment type. Thus, utilization of two or more species from different major taxa increases the probability of accurately assessing the maximum field tissue residues.

The actual number of species and taxa used depends upon the goals and scale of the project and the range of pollutants in the sediment. In general, use of a single species should be adequate for a general survey of an area or assessing a small discharge or volume of dredge material. In interpreting the data from a single species test, however, it should be recognized that no one species is likely to maximize uptake from all pollutants. Two species are recommended when assessing a moderate to large sized discharge or dredging operation. The species should be of different major taxa, and a polychaete and a bivalve are recommended. It is especially important to include a bivalve if PAH contamination is of concern, as bivalves have a reduced capability to metabolize PAHs compared to amphipods or polychaetes (Varanasi, et al. 1985). The addition of an arthropod species or additional polychaete and/or bivalve species may be justified when assessing a large discharge or dredging operation, especially if there is a wide range of pollutants.

CHAPTER VIII: ORGANISM COLLECTION, MAINTENANCE, TRANSPORT, AND ACCEPTABILITY

To assure unbiased test results, bioassay organisms must be in good health and have minimal background pollutant contamination. Reasonable efforts must be taken to minimize stress during collection and transport to holding facilities. Holding facilities must provide high quality water and conditions suitable for the maintenance of the test species. This chapter describes techniques and facilities which will meet these general requirements.

A. ORGANISM COLLECTION AND TRANSPORT

1. Field Collection of Test Organisms

The logistics of collecting intertidal species is usually much simpler than those of collecting subtidal species, and intertidal collection is recommended when possible. Infaunal organisms can be collected by turning the sediment over with a shovel and picking out larger species (e.g., clams) or by gently sieving the sediment in the field. For most of the bioaccumulation test species, a sieve size of 4-6 mm will collect adequate numbers while minimizing damage and sorting time. Collection equipment should not have been used in contaminated sites or should have been adequately cleaned.

Subtidal organisms can be collected by grabs, dredges, or suction samplers (see Holme and McIntyre, 1984). Dredges sample a larger area than grabs and are usually more proficient at collecting shallow-buried organisms, though there is a greater

possibility of damaging specimens. Grabs are recommended for collecting more deeply buried species. Suction lifts are also useful for collecting larger, deeply buried bivalves, though they require the use of SCUBA divers, and a greater likelihood of damage exists. Electro-shocking, chemical poisons, and other harsh methods of collection are not acceptable.

Remove organisms from the collection device as soon as possible and submerge them in ambient seawater or sediment contained in ice chests or uncontaminated plastic buckets. Avoid overcrowding animals in collection containers. Discard organisms with signs of disease or obvious defects (e.g., bivalves with cracked shells).

State or local authorities may require collection permits or ban collection from specified areas. Collection of regulated species (e.g., bay clams) may require a local license, be limited to a season, and preclude certain collection techniques. Additional permits or precautions may be required when importing non-indigenous species. Check with State authorities about the local regulations before collecting or importing specimens.

2. Organism Transport

For organism maintenance, ASTM (1984) recommends not more than a $3^{o}C$ change in water temperature within a 12 hour period and an oxygen concentration of between 60 and 100% of saturation. If the time between collection and return to laboratory is short (less than 1-2 hours) and ambient temperature is not extreme, simple precautions should meet these requirements. If possible,

collection buckets or ice-chests should be kept out of direct sunlight and must not be left in closed vehicles. The water in the containers should be changed periodically while collecting and immediately before returning. If the time before returning to the laboratory is several hours and/or air temperature is high, use a portable aerator to maintain oxygen levels.

Successful long-distance transport of organisms, whether in a vehicle or through the mail, requires packaging that retains moisture and maintains an adequate supply of oxygen. This can be accomplished by placing animals in a minimum amount of water (a few milliliters) in a sealed container filled with air (e.g., Whirl-Pak^{R} bag). Alternatively, test animals may be placed between wet nylon or seagrass (e. g., Zostera) and surrounded by layers of wet paper towels, all contained in polyethylene Ziplock^{R} bags (Robinson, A., pers. comm. AScI, Mar. Sci. Ctr., Newport, OR; Gulf Specimen Co., pers. comm., Panacea, FL). Wet sediments may also be used to retain moisture. These sediments should have a low organic content (e.g., ashed sediment, beach sand) as they are not as likely as natural sediment to turn anoxic. Regardless of the moisture retaining agent, the container should have a large air space to maintain aerobic conditions. Air trapped within a plastic bag has the added advantage in preventing animals from being crushed.

Containers with organisms should be placed in ice chests or insulated shipping containers, with packets of jelled (e. g., Blue Ice^{R}) refrigerant placed at or taped to the inside of the top

of the container. Jelled refrigerants are preferred to ice to avoid melt water and a layer of insulating material should be placed between the refrigerant and animals. Add sufficient refrigerants to maintain the water temperature in the containers at or a few degrees below the water temperature at the collection site but not so much as to cold shock the organisms. Insulating material should fill all extra space in the shipping container, protecting and securing the bottles and bags in the carton. Pack shipment containers to obtain a low center of gravity, and label plainly to keep package upright. Every effort should be made to provide overnight or 24-hour delivery. If the organisms are transported by vehicle, periodically monitor the temperature and drain any melt water and replace the ice as required.

B. CULTURING AND PURCHASING TEST ORGANISMS

A successful culture of an appropriate test species has the advantages of providing a ready supply of specimens with a known history. However, culturing of marine/estuarine organisms is not a task to be undertaken lightly, and is usually justified only if regular tests are planned. Although a few sediment ingesting polychaetes can be cultured with relatively simple equipment (see Appendix VII-1), the majority of recommended test species are not routinely cultured.

Some test organisms can be purchased from biological supply houses, local collectors, colleges, or bait shops. Purchasing specimens can be cost-effective if the laboratory is not well

equipped for collection (e.g., does not have a boat) or if tests will be conducted only occasionally. There are several companies that specialize in supplying bioassay organisms (see Appendix VIII-1), although most do not presently supply appropriate benthic bioaccumulation organisms on a routine basis. Check with a supplier even if bioaccumulation test species are not currently carried as availability of particular species may change or the supplier may be able to fill special orders.

Maintain purchased organisms in the laboratory for at least one week to acclimate them to local conditions and to monitor their health. Before beginning bioaccumulation tests, analyze the purchased organisms for background pollutant levels to determine if they meet the criteria for control organisms (see Table VIII-1).

C. PRE-EXPERIMENTAL MAINTENANCE

In general, the guidelines presented here are based on our experience with Macoma nasuta or modified for deposit-feeders from ASTM Standard Procedures (ASTM 1980, 1984) for maintaining filter-feeding bivalves for bioconcentration tests. Most of the bioaccumulation test species are adaptable to laboratory conditions, so elaborate procedures are not usually required for the maintenance of adults. Additional information on the maintenance of benthic invertebrates can be found in King and Spotte (1974), Dean and Mazurkiewicz (1975), Kinne (1976-1977), and National Research Council Committee on Marine Invertebrates (1981).

When maintaining a non-indigenous species, permits may be required from state or local authorities. This may require fail-safe precautions against accidental release of such organisms into the local environment (i.e., double containment, diked seawater drains, siphon breaks, etc.). Equipment, water, wastes, and dead animals may require sterilization before disposal.

1. Water Quality

Constant water quality should be maintained in holding aquaria, keeping dissolved oxygen between 60-100% saturation and un-ionized ammonia concentrations <20 ug/L (ASTM, 1984). Flow-through seawater with a minimum flow-rate of 1 L/hr per gram of wet tissue is recommended as a means of maintaining water quality. Filtering incoming seawater is generally inadvisable with benthic invertebrates as the settling particles supply a natural source of food. If flowing seawater is unavailable, organisms can be maintained in static systems using collected seawater. Store replacement seawater in covered containers in the dark at 4^{o}C to keep salinity and water quality constant. If collection of natural seawater is impractical, artificial seawater may be used, though it should be demonstrated that the growth and behavior of the test species is not altered by using artificial salts. Prepare artificial seawater with deionized water or with distilled and charcoal-filtered water. Static systems should always be aerated, and preferably equipped with a recirculating aquarium filter with replaceable activated charcoal.

Regardless of whether flow-through or static systems are used, the seawater should be analyzed for background levels of pollutants, especially if it is collected from an urbanized area. If a pollutant is detected in the water, its potential uptake can be estimated by multiplying the water concentration by the bioconcentration factor (BCF) for that compound. If the calculated tissue residue is greater than that acceptable for a control organism (Table VIII-1), a different water supply is required. BCF values and methods to estimate BCFs can be found in Bysshe et al. (1982).

Temperature should not vary more than 3^{o}C in a 12 hour period and salinity should not vary by more than 2 g/kg or 20% of the average, whichever is larger (ASTM, 1984). In flow-through systems, a storage tank within the laboratory will help ameliorate natural fluctuations in temperature. In estuarine areas, a storage tank may be necessary to supply high salinity water during low salinity periods.

2. Sediment Quality

Maintain animals in a sufficient amount of clean sediment to allow them to burrow naturally, which in nearly all cases will be at least 3 cm. This sediment must be analyzed for pollutant concentrations, which should not exceed the level acceptable for a control sediment (Table II-1). Periodically add fresh sediment of the same type to maintain an adequate food supply (i.e., detritus and associated microbes). One to three times a week, add about two millimeters of fresh control sediment to the

sediment surface. This sediment replenishment should be sufficient if the organisms are not over-crowded. If the sediments become heavily pelletized with fecal material, remove the organisms and replace the sediment. The addition of other types of food are not recommended except in special cases of long-term maintenance. These foods include detritus or decaying seaweeds, cultured marine phytoplankton and zooplankton, microencapsulated diets, formulated feeds such as fish flakes (e.g., TetraMin^{R}), or small bits of clam or other tissues for omnivores (Lee and Muller, 1972). Check the background pollutant levels of all foods.

3. Organism Health And Acclimation

Field collected organisms should be held in the laboratory for at least four days before commencing a bioassay, and purchased organisms held for at least a week. Discard any organism if injured or behaving abnormally. In general, animals should not be held longer than two weeks before testing. If longer maintenance periods are needed, the investigators should have experience with the species and should monitor for any signs of stress (e.g., reduced sediment processing rate, unusual tube construction). A flow-through system is strongly advised if long-term maintenance is planned.

To avoid the spread of diseases, organisms collected more than a week apart should be maintained in separate aquaria, each with an independent water supply. The organisms should be checked every day or so, and any diseased, dying, and dead

organisms promptly removed. Black spots on the surface of the sediment can mark the location of dead organisms. Should a question arise concerning the health of the animals, a behavioral test such as time to rebury or analysis of lipid content is recommended.

If holding and experimental conditions are different, gradually acclimate the test organisms to experimental conditions. This transition may be accomplished using serial water dilutions until the proper temperature, salinity, and pH are reached. Acclimation for temperature should proceed no faster than $3^{o}C$ in 72 hr (ASTM, 1984). Maintain animals at the test temperature and salinity for at least two days before commencement of an experiment. No more than 3% mortality is permitted within 48 hr before the test (ASTM, 1984).

D. ORGANISM ACCEPTABILITY AND BACKGROUND CONTAMINANT LEVELS

Specimens selected for a test must tolerate the physical-chemical conditions (e.g., TOC content, interstitial salinity) of the test substrate and be free of disease or stress from capture or handling. All specimens should be collected from the same site, and preferably at the same time. All organisms used in a given test should be as uniform in age and size as possible, and bivalves should be of the same year class. For bioconcentration tests, ASTM (1984) stipulates that the length (umbo to distal valve) of the largest clam should be no greater than 1.5 times larger than the smallest clam. For Macoma nasuta, this would be

equivalent to a 3.7-fold difference in wet flesh weight. It is important to correctly identify the test species, and voucher specimens should be kept from each collection.

High pollutant background levels in the test specimens may confound the results, making it difficult to detect differences between treatments. Therefore, tissue residues in the test organisms should be no greater than those expected in organisms living in control sediment (see Chapter II). Approximate acceptable background tissue concentrations for test species are given in Table VIII-1. These values are from organisms collected from sites which appeared to meet the criteria for a control site. For compounds not listed in Table VIII-1, the ASTM (1984) criterion of the background tissue residue not exceeding 10% of the expected steady-state can be applied. First-order estimates of steady-state tissue residues can be obtained from data on other species or from the thermodynamic-based bioaccumulation model for neutral organics (see Appendix I-1).

TABLE VIII-1: Representative Control Organism Tissue Residues

COMPOUND			
Organics (ppb wet wt.)	Various East Coast Sites[a]	Puget Sound[b]	Yaquina Bay, OR[c]
CB	<1.0-70		
B(ibk)F		<10	
BaP	0.3-6.0*	2.3-<10*	1.9
DDT	<0.08-3.8	<1.0-<5.0	3.9
HCB	0.02-0.17	<130	
Naph	<1.0-9.1	<0.05	
PAH	0.02-7.2	<2-17*	
PCB	10-70	<2.0-10	
pesticides	<0.03-0.6		
Metals (ppm wet wt.)	Various East Coast Sites[a]	Puget Sound[b]	Yaquina Bay, OR[c]
Ag	0.2-2.6		
As	1.5-3.9		
Cd	<0.06-4.0		<0.005
Cr	0.26-2.5		
Cu	0.1-7.2		<1.5
Hg	<0.05-1.2	1.0	
Ni	<0.4-7.0		
Pb	<0.6-2.6		
Zn	2.4-30		<2.0

a = Tetra Tech, 1985a
b = Konasewich et al., 1982
c = unpublished data
* = Tetra Tech, 1985a and Konasewich et al., 1982

CB - Chlorinated benzenes
B(ibk)F - Benzo(i,b,k)fluoranthene
BaP - Benzo(a)pyrene
HCB - Hexachlorobenzene
Naph - Naphthalene
PAH - Polyaromatic hydrocarbons
PCB - Polychlorinated biphenyls

CHAPTER IX. SEDIMENT EXPOSURE SYSTEMS

In designing the exposure systems, considerations for maintaining an adequate environment for the test organisms must be combined with considerations for pollutant behavior, costs of construction and maintenance, and ease of operation. The recommendations made here are based on the assumption that each "exposure chamber" will hold a single experimental unit (e.g., individual organism or composite of a single species in a beaker), but that these may be placed into a larger aquarium or tank to maintain water quality. The "exposure system" is composed of the replicate exposure chambers, any aquaria or tanks which hold the exposure chambers, the water delivery system, and any pollution abatement system. The recommendations are also based on the standard 28-day exposure duration. Discussion of specialized exposure chambers is limited to Appendix IX-1.

A. SYSTEM REQUIREMENTS

In designing the exposure system, two critical considerations are the amount of sediment per individual and the volume and turnover rate of water. Adequate amounts of sediment and overlying water are required to assure that supplies of food and pollutants are not substantially depleted, water column oxygen concentration is not depressed, metabolites are diluted, and the organism's feeding behavior is not impaired. The ASTM criteria for bioconcentration (ASTM, 1984) and toxicity tests (ASTM, 1980) offer general guidance to the design of a sediment

exposure system, but several of their specific recommendations had to be modified because of the different requirements for deposit-feeders compared to fish and filter-feeders. Additionally, the design criteria for maintaining a constant pollutant concentration in the water are inapplicable as the sediment instead of water is the exposure mode in these tests.

1. Sediment Requirements

Sediment serves as the habitat, source of food, and source of pollutants for the test organisms. If insufficient sediment is added, organisms may reingest the same particles. Alternatively, if the fecal pellets are resistant to breakdown, there may be a reduction in the appropriately sized particles, especially with the more selective deposit-feeders. Both of these processes could reduce the mass of pollutant available to the test organisms. Although both reingestion and pelletization of sediments occurs in field (see Lee and Swartz, 1980), the rates may be exaggerated in laboratory systems. For this reason, it is critical to supply a sufficient sediment mass during the entire course of the laboratory exposure.

Assuming periodic sediment additions to the exposure chambers (see Chapter X), we recommend initially adding at least 50 grams wet sediment for each 1 gram wet flesh tissue (excluding shell) for surface deposit-feeding bivalves. For funnel-feeders such as arenicolid worms, at least 200 grams of wet sediment to each 1 gram of wet flesh tissue may be required for construction of a normal feeding burrow. Besides the mass of sediment, the

sediment must be deep enough to allow normal burying and feeding. The initial depth for Macoma should be at least 2 cm and preferably 3-5 cm, whereas a large lugworm may require 5-10 cm of sediment. While these amounts should bracket the needs of most organisms, the initial amount of sediment added should equal or exceed consumption requirements for the 28-day exposure. For example, if a particular species processes 2 grams of sediment per gram of tissue per day, then at least 56 grams per gram of tissue should be added initially. A more accurate estimate of sediment requirements for selective deposit-feeders can be generated by using the processing rates of the ingested size fraction, though this information will not usually be available.

If periodic sediment additions are not made, then the initial amount added should exceed the total amount processed over the duration of the experiment by at least 2-fold, and preferably 5-fold. Thus, for the organism with a 2g/g-tissue/day sediment processing rate, about 250-300 grams of sediment should be added per gram of tissue. Compilations of sediment processing rates (e.g., Lee and Swartz, 1980) can be used to estimate these requirements. It must be recognized, however, that in a laboratory, an organism may deplete the food or pollutants within its specific feeding zone regardless of the amount of sediment added. This is especially likely with surface deposit-feeders. For this reason, we strongly recommend periodic additions of the treatment sediment, as discussed in Chapter X.

2. Water Quality

The water quality requirements for conducting the tests are similar to those for pre-experimental maintenance (Chapter VIII). That is, dissolved oxygen should be between 60 and 100% of saturation and the un-ionized ammonia concentration should not exceed 20 ug/L (ASTM, 1984). These criteria can be met either by using a flow-through system or a static batch-replacement mode.

For a flow-through system, ASTM (1984) recommends not more than one filter-feeding bivalve (40-60 mm from umbo to edge of distal valve) per liter per hour. This would be equivalent to about a minimum flow of one L/hr/g wet tissue for an oyster. However, this requirement is based on feeding, and does not account for sediment oxygen demand. In addition to the flow rate per gram tissue, flow-through systems should be designed to achieve at least six turnovers a day.

In static systems, the water volume to loading ratio needs to be sufficient to allow the maintenance of oxygen levels $\geq$60% of saturation. A gentle aeration helps maintain the oxygen level as does changing the water two or three times a week. As an example, 10 Macoma nasuta (mean wet flesh weight of about 1.3 g), each in a 100 ml beaker with an initial 50 grams of sediment, have been successfully maintained in a 10 L aquarium with 8 L of filtered seawater (Ferraro et al., 1990). The aquarium was gently aerated and the water changed three times a week.

In determining the oxygen demand for the system, it is important to take into account the total sediment oxygen demand.

In most cases, sediment microbial demand will be several fold greater than the oxygen utilization by the test species. Therefore, calculations based on sediment-free exposure systems will underestimate the actual oxygen requirements. The total oxygen demand of sediments ranges from <1 to over 100 ml $O_2/m^2/hr$ (e.g., Hargrave, 1969; Smith et al., 1973; Smith, 1978; Davis and Lee, 1983). In general, total oxygen demand will increase with temperature and in organically enriched sediments, and the water flow or volume should be increased accordingly.

Aeration will help ensure a proper oxygen concentration is maintained, and is required in a static system. The air should be filtered and free of oil and moisture. The volume should be sufficient to turnover the water but not enough to resuspend the sediment. This can be achieved with a supply of approximately 0.1 SCFH (standard cubic foot per hour) per 10 liter aquarium via an air stone or pipette. Position the air stone or pipette outside of beakers maintained in aquaria, or sufficiently far above the surface to avoid resuspension in individual beakers or aquaria. Check the air-stone or bubbler frequently and remove any salt crystals forming at the orifices. If air is provided from a compressed air tank, specify that the composition include about 0.3-1.0% CO_2 to help control pH. If not specified, no CO_2 will be present.

Seawater is well buffered, but in static systems metabolites and waste materials (i.e., ammonia) can build up, lowering pH. Maintain pH between about 6.5 and 8.0 (ASTM, 1984). As mentioned,

aeration will help maintain pH as will periodic replacement of water. In static systems, the addition of clean, crushed oyster shell to the bottom of the aquarium can provide a good buffering system.

ASTM (1984) recommends that overlying water salinity should vary less than 2g/kg or 20% of the average, whichever is higher. In areas where salinity varies (as in water drawn from estuaries with a seasonally high riverine contribution), store a quantity of high salinity water sufficient for the expected period of low salinity, or preferably, to maintain salinity over the duration of the exposure.

Because phytoplankton and suspended material are a sink for pollutants and a food for facultative filter-feeders, it is important to filter the water to remove suspended particulates (>5 um) during the test. Filtration can be accomplished with in-line cartridge filters (commercially available with 2.5-5.0 um pores) or in batch mode. The ASTM (1984) precautions concerning the adequate concentration of phytoplankton necessary as a food source are not relevant for deposit-feeders.

3. Temperature and Light

The tests should be conducted as close as possible to one of the seven temperatures recommended by ASTM (1984) - 7, 12, 17, 22, 27, and 32^{o}C. A temperature corresponding to the average spring-summer temperature of the study site would simulate the biologically most active season. Most commonly, this will be 12^{o}C in the Pacific Northwest, 17^{o}C in mid-latitudes, and 22 or

27°C on the Gulf Coast. With flow-through systems, it can be difficult to exactly adjust the temperature, though a large head tank in a controlled-temperature room will temper the water. The maximum difference between the minimum and maximum temperatures must not exceed 10°C (ASTM, 1984).

Light should be provided by means of cool-white fluorescent lights at an intensity of about 400 foot-candles. Other sources (incandescent, fluorescent/incandescent, photosynthetically active radiation augmented) may be required for special purposes. Ultraviolet radiation, especially UV-B, is generally missing from these artificially supplied spectra. Although UV-B radiation can enhance the toxicity of certain pollutants (phototoxicity), this probably will not greatly affect organisms buried in sediment. ASTM (1984) recommends a 16h day, 8h night as a convenient light/dark cycle. Schedules of 12/12 or 14/10 hrs day/night are also acceptable, and may be useful in delaying maturation and spawning of some species. We have routinely used a 12/12 schedule. These various day/night cycles can be controlled by use of timing devices in the light circuits.

B. EXPOSURE SYSTEM DESIGN

1. Materials Compatibility

Materials used in the exposure system should not induce any reaction by the organisms or affect pollutant concentration or bioavailability. Borosilicate glass (Pyrex^{R}, Kimax^{R}, or equivalent) and soft glass (soda-lime, window) have proved generally non-reactive to metals and organics, and are the

preferred materials where their fragility is not a major limitation. Most rigid plastics (polyolefins, engineering resins and fluoropolymers) are acceptable after conditioning, such as soaking in deionized water for several days. Some plastics, generally flexible types that contain mobile plasticizers (phthalate esters), need to be tested for toxicity and pollutant compatibility. These flexible plastics should not be used if the uptake of phthalate esters will be studied. Because the alloy components of many stainless steels may react with saltwater, stainless steel should not be used in direct contact with seawater. Choose another material if pollutant sorption to internal surfaces of containers is a problem.

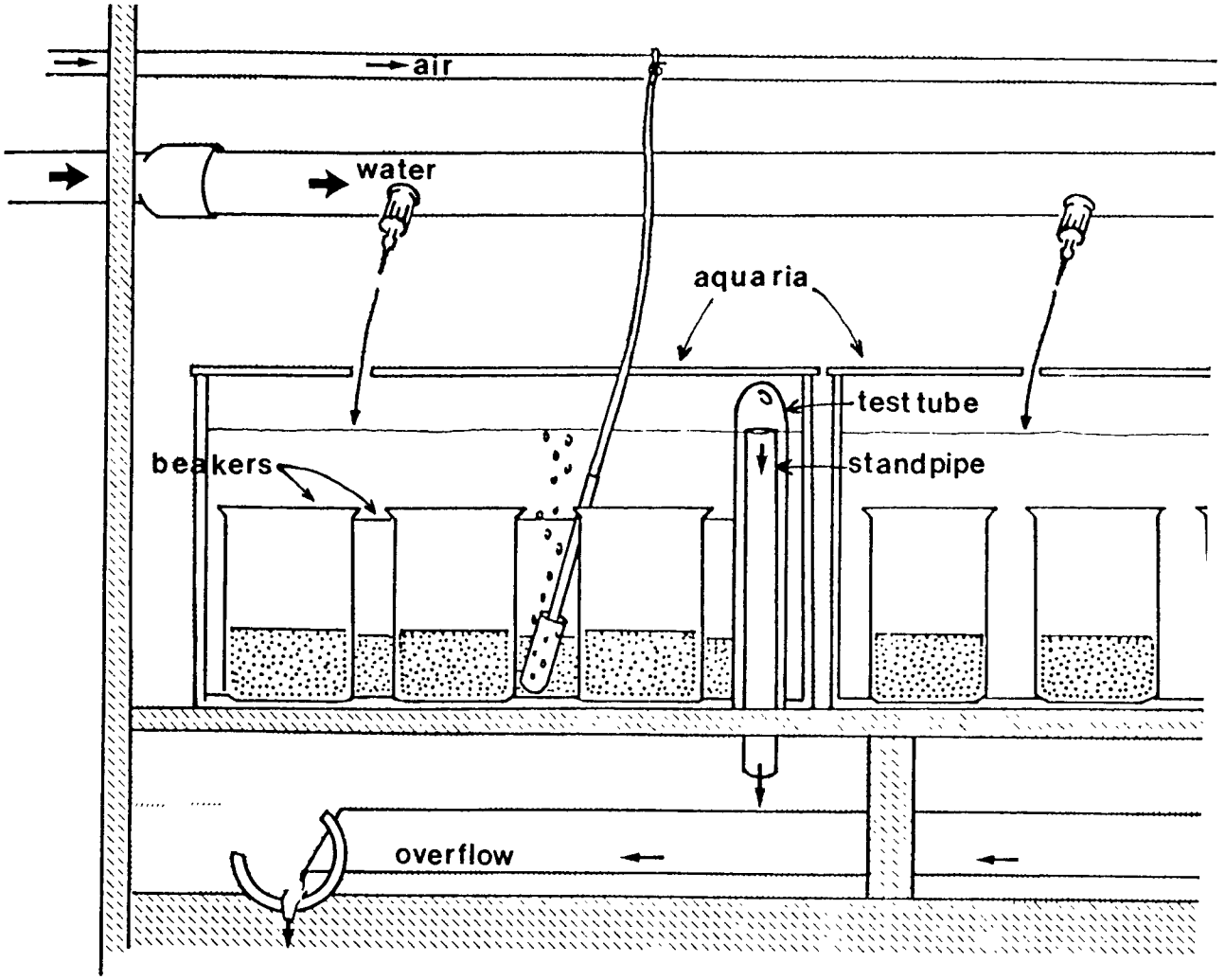
Any sealant used to construct chambers must be non-toxic, such as Dow-Corning^{R} #8641 clear, non-toxic silicone-rubber (i.e, meets FDA Regulation 21 CFR 177.2600). Such materials are usually specified for aquarium use and do not contain fungicides (e.g., arsenic compounds). Exposed sealant at joints should be minimal. Place sealant used for mechanical reinforcement on the outside of the joint. Plastics and sealants must be chosen carefully, as both may sorb pollutants. Product literature on the material is helpful in determining the compatibility of a particular plastic to a pollutant.

2. Exposure Chamber

The actual exposure chamber can consist of glass boxes, beakers, aquaria, or other containers of appropriate material (see Figure IX-1). For most species, beakers are an inexpensive

FIGURE IX-1

Representative Sediment Exposure System



exposure chamber. The diameter of the beaker and the sediment depth in the beaker should be sufficient to allow the organism to bury and/or construct normal tubes. With Macoma nasuta, about 50 mm diameter beakers (Corning #1000, 100 ml) with about 50 g of sediment were sufficient for 25-32 mm clams (anterior to posterior measurement) to bury normally. With 35-48 mm clams, 68 mm diameter beakers (Corning #1000, 250 ml) with about 100 g of sediment were required. In both cases, the initial depth of the sediment was about 3-5 cm depending on sediment type. If the beakers are placed into an aquarium, the beaker height should be several centimeters less than the water height to allow for circulation into the beakers. For funnel-feeding arenicolid worms, a long narrow glass box (about 1"w, 7"l, 4.5"h) is a more appropriate shape (see Figure IX-1B). The opening of the exposure chamber should allow the periodic addition of feeding sediment.

3. Static Exposure System

The simplest static systems are individual aquaria or beakers filled with water as commonly used in sediment toxicity tests (e.g., Swartz et al., 1985a). A more common design for bioaccumulation tests are sets of beakers submerged in aquaria (e.g., Ferraro et al., 1990). The beakers or aquaria should be covered to reduce evaporation and gently aerated to maintain dissolved oxygen levels. Drain tanks about once every two days by siphoning water from the aquaria, but not the individual beakers. Retain the waste water in a container for treatment if

it is suspected of containing unacceptable pollutant levels for disposal down the sanitary sewer. Gently refill the aquarium with water of the correct salinity and temperature, and restart the aeration.

4. Flow-Through Exposure Systems

For flow-through systems, chambers may be sets of beakers maintained in aquaria (Figure IX-1) or entire aquaria. Flow-through systems have the advantages of removing waste products and maintaining oxygen. Though desirable, a flow-through design is not normally required for a successful bedded sediment test. To avoid cross contamination, water flowing through one container must not flow into another container. Water exiting the systems should be passed through a charcoal filter if substantial desorption of pollutants from the sediment is anticipated. Similarly, resuspended sediment should be trapped and retained as waste. Examples of conducting flow-through tests can be found in U.S. EPA (1978), Rubinstein et al. (1980), and Rubinstein et al. (1987).

C. MULTIPLE SPECIES EXPOSURE CHAMBERS

If several species are being tested, it is possible to place multiple species within each exposure chamber. The advantages of multiple species per container include reduced space requirements and a lower cost because of the reduction in the number of chambers constructed and maintained. The greatest disadvantage is the potential for negative interactions among the species,

such as competition or predation, which could have unknown and variable effects on uptake. For example, uptake could be reduced in Macoma if siphon clipping by epibenthic shrimp or nereid worms reduced the clam's feeding rate. Another disadvantage is that the accidental loss of a chamber reduces the number of replicates for each of the species.

If multiple species are placed within exposure chambers, the amount of sediment initially added should at least equal the sum of the amount required for each individual species. Most of the potential interactions are density dependent, so increasing the area of the chambers (while maintaining a sufficient sediment depth) should reduce the intensity of any negative interactions. An alternate design is the physical partitioning of the aquarium with screens to separate the species (e.g., Rubinstein et al., 1987).

Regardless of the specific design, the same numerical ratio of one species to another must be maintained in replicate chambers. It should also be noted that a paired-comparison approach should be used when statistically comparing the tissue residues of two species kept in the same chambers.

CHAPTER X: EXPERIMENTAL INITIATION, MAINTENANCE AND SAMPLING

A. EXPERIMENTAL INITIATION AND MAINTENANCE

1. Pre-Experimental Preparations

Coordinate the collection and acclimation of the bioassay organisms with the collection of the sediments so the experiment may begin with a minimum of delay. The glassware, water delivery system, and any stored water should be ready, as well as sampling containers, labels and related paraphernalia. Beakers and other containers should be pre-labeled. A detailed work schedule, showing daily tasks and persons responsible for accomplishing them, should be prepared before the sediment arrives. A prearranged numbering scheme should be agreed upon with the analytical chemists. It is critical to keep the analytical chemists well informed of the sampling schedule so they can prepare for the sample load. Arrange with maintenance personnel to look for power failures, pump leaks, breakage of aquaria, inadvertent switching on of lights at night, and other accidents. Provide telephone numbers for key personnel responsible for maintenance of the experiment in a prominent location (e.g., on the door of the laboratory). Any safety warnings should also be posted at entry points.

2. Experiment Initiation

Weigh all individual organisms or composites of organisms, while taking care to minimizing exposure of soft-bodied organisms to the air. To avoid temperature shock, maintain the air temperature of the room at the experimental water temperature.

All bivalves should be measured (anterior to posterior valve), weighed, and individually marked with a random number. When possible, the number on the clam should be the same as the number on their exposure chamber. Clams can be marked with a laboratory marking pen (e.g., Sharpie^{R} pen) by first scrubbing the shell with a Kimwipe^{R} or other soft paper towel, blotting the shell, and then allowing them to dry (about 15 minutes at 12^{o}C). Mark the same valve (i.e, right or left) in all clams. Discard any organisms not meeting the criteria for size or condition. Maintain some extra individuals for potential replacements within the first 24 hours. Also, randomly choose some specimens for wet-to-dry weight conversions and for long-term storage for potential lipid analysis with a different technique (see Chapter XI).

Distribute measured aliquots of homogeneously mixed sediment to each exposure chamber. Weighing the sediment aliquot is preferable, but sediment volume can be used to estimate mass for a particular sediment type. During the process of measuring out aliquots of sediment, periodically re-stir the source to avoid separation of the fines and interstitial water. If beakers are used as the exposure chamber, gently tap the beaker to consolidate the sediment and eliminate air bubbles. To avoid the loss of surficial fines when filling the beakers, place a plastic film over the sediment surface, slowly fill the beaker with water, and then withdraw the film using forceps. Carefully place the water-filled beakers into filled aquaria and allow any suspended fines to settle.

If aquaria or other large containers are used as the exposure chambers, stir the sediment after adding the appropriate amount to mix sediment and remove any bubbles. As with the beakers, a plastic film should be placed over the sediment surface when filling the aquarium with water. Position any aerating device so that the induced turbulence does not resuspend sediment.

Add the organisms after allowing the sediments to consolidate and any suspended particles to settle, which will normally take from 15 minutes to a day. Place animals on the surface of the sediment and allow them to bury. To facilitate burial, place Macoma nasuta left valve down ("bent-nose" up) on the sediment. Mobile organisms, such as the polychaetes, should be observed for a sufficient period to assure that they bury in the correct chamber and do not swim into another chamber. For mobile worms, it may be necessary to place a screen on the tops of beakers to keep them from swimming out.

2. Experiment Maintenance

Replace any animals whose behavior is abnormal (failure to bury in the sediment, etc.) within the first 24 hours. Observe the chambers daily and note any signs of abnormal activity (e.g., reduced production of fecal pellets, avoidance of the sediment). Remove any beakers with dead organisms. It is especially important to monitor for dead organisms in a static system. Record temperature, salinity, and other water quality parameters on a periodic basis (see Chapter IX). Replenish water in static

experiments according to a preplanned schedule (Chapter IX), and dispose of drained water in accordance with applicable rules for hazardous waste.

3. Sediment Renewal

We strongly recommend periodic additions of small amounts of the appropriate sediment type to each exposure chamber. Because the bioavailable fraction may only be a small portion of the total sediment-associated contaminant (see Landrum, 1989), it is possible for organisms to deplete the available fraction, especially in organisms which ingest sediment from a restricted feeding zone (e.g., surface deposit-feeding bivalves). For example, depletion of the bioavailable fraction may be the reason that tissue residues of 35 of 37 compounds declined between day 39 and day 79 in Oliver's (1987) study of uptake by oligochaetes. Also, without organic input from settling phytoplankton and with low light levels inhibiting benthic microalgae, it is possible for the nutrient quality of the sediment to decline over the course of an experiment. Periodic sediment renewal should reduce these potential laboratory artifacts and help maintain a more constant pollutant concentration and food supply. The periodic addition of sediment results in a pulsed-renewal exposure. Without the addition of new sediment, the exposure is a single-dose exposure.

The daily amount of sediment added should equal or exceed the daily sediment processing rate of the organism. Sediment ingesting clams such as Macoma require about 1 gram wet sediment

per gram of wet tissue mass per day while arenicolid worms (2-6 g wet weight) require about 10 g of sediment per day. It is sufficient to add the sediment two or three times a week (e.g., about 3.5 g twice a week for a 1 gram Macoma). Previously, we had frozen the sediment immediately before its addition to reduce loss of fines (Specht and Lee, 1989; Boese et al., in press); however, given the unknown effects of even short-term freezing, we recommend adding the sediment using a cut-off plastic syringe. We have successfully used a 3 cc syringe with both a fine sand and a silt-clay. The volume of sediment in the syringe is a simple way to estimate mass of sediment, though the volume to weight ratio has to be determined for each sediment.

For long-term exposures (>28 days), we recommend periodically replacing all the sediment in the chambers. Replacement of sediment reduces the possibility of depletion of the bioavailable fraction of the pollutants and/or food, and avoids excessive pelletization of the sediment. Additionally, the periodic addition of surface sediments will overfill most chambers within a few weeks, requiring a complete replacement of the sediment. Replacement on a 28-day schedule should suffice, and coordinates with the long-term sampling schedule (see Chapter IV). If a field sediment is being tested, all the sediment should be collected at the same time and the renewal sediment stored until needed. If a spiked sediment is being tested, it may be preferable to spike new sediment for replacement.

Do not feed the test organisms a supplemental source of food in either 28-day or long-term experiments. By ingesting the added food, the organisms are presumably ingesting less sediment, which could result in an underestimation of the bioavailability of the sediment-associated pollutants. The addition of food is not required as shown by long-term maintenance (>28 days) of deposit-feeding bivalves (e.g., Specht and Lee, 1989), polychaetes (e.g., McElroy and Means, 1988), and crustaceans (e.g., Landrum, 1989) without supplementing the sediment with an artificial food source.

B. SCHEDULE FOR ABIOTIC AND BIOTIC POLLUTANT SAMPLES

Samples of sediment, water, and biota should be taken for pollutant analysis before, during, and after testing. Sampling techniques and apparatus may vary with the nature of the sediment, species of test organism, and compound(s) of interest. As the manner in which samples are taken may affect the analysis, consistency in sampling for any given parameter is essential.

1. Overlying Water

Although no pollutants are intentionally added to overlying water in sediment bioaccumulation tests, contaminants may be introduced from the water supply system, leached from the sediment, or present on resuspended particulates. The activities of some species (e.g., Yoldia) can resuspend considerable amounts of fine-grain material directly into the water column. With a randomized block design (Figure III-3f), bioturbation may lead to cross-contamination between treatments. This potential uptake

from the water needs to be quantified to differentiate it from uptake from the bedded sediment and to check for possible cross-contamination among treatments.

At a minimum, overlying water should be sampled for pollutants from each treatment at the beginning, middle, and end of the test period (i.e., T_0, T_{14}, and T_{28}). A sample from each aquarium should be analyzed if statistical comparisons are planned, though in many cases it would be acceptable to composite water samples from aquaria of the same treatment. If samples are composited, individual samples from each aquarium should be archived in case a more detailed analysis is required. Samples should also be taken during periods of high turbidity or other unusual water quality.

Overlying water should be sampled at mid-depth from each exposure unit. When experimental units share the same overlying water (e.g., test beakers within the same aquarium), overlying water should be sampled from mid-depth of the entire container. Care should be taken to avoid disturbing the flocculent material at the sediment-water interface. Sampling apparatus (pipettes, sample vials) should be made of materials that do not appreciably absorb or leach pollutants. To guard against cross-contamination, rinse off the sampling apparatus after each use. Sample volumes will depend upon the analytical technique used, but may range from about 1 to 100 ml.

2. Sediment and Interstitial Water

Sample all test, control, and reference sediments before the addition of organisms (t_0 sample) and at the end of the exposure (typically t_{28}). These sediment samples should be analyzed for pollutant concentrations, TOC, and moisture content. In most cases, it is adequate to conduct the grain size analysis only on the initial sample.

One procedure for sampling sediment for organic pollutants from exposure chambers is as follows:

1. Remove overlying water from the exposure chamber by siphoning or decanting, taking care not to disturb the surface floc. Depending on the procedure, interstitial water samples may be taken at this stage.
2. Remove the test organism(s) from the sediment. Larger bivalves can be directly removed with forceps. Spread the sediment out in a tray to remove small bivalves and polychaetes. Do not use any water to remove the sediment from the exposure chambers.
3. Homogenize the test sediment from each exposure chamber by stirring with a Teflon^R coated spoon, glass rod, or other inert utensil. Take a sediment sample from each exposure chambers, place in a labeled sample vial and freeze. These individual samples will either be analyzed or archived if composites are analyzed.
4. If composites are going to be taken, the compositing strategy will depend upon how the exposure chambers were

allocated among aquaria. If only one treatment type is placed in each aquarium, composite all the beakers within an aquarium. If the exposure chambers are allocated randomly among aquaria, combine all the sediment from each treatment (i.e., sediment type) regardless of aquarium. In both cases, homogenize the sediment, take replicate samples from each composite, and freeze until analyzed.

Extra sediment samples should be taken from individual exposure chambers (and from any composites) and frozen in case there is an analytical failure or greater statistical power is required.

Because this procedure exposes the sediment to the air, reduced metal forms will be oxidized. If metal speciation will be studied, the procedure should be modified, especially steps 2 and 3, to minimize the sediment's exposure to air. One possibility is to take small sediment cores from the exposure chambers. Regardless of sampling scheme, interstitial water should be collected at the same time as the sediment samples. Interstitial water may be collected by a variety of methods including centrifugation, sediment squeezing, and dialysis membranes (see Chapter VI).

C. METHODS OF BIOTIC SAMPLING

Test organisms need to be carefully removed from the sediment, as described above, and all adhering particles removed. A gentle rinse with clean seawater will help remove particles from polychaetes. In general, organisms should be placed in

control sediment to purge their gut contents for 24 hours before chemical analysis (see Section D). At the end of the purging duration, collect the organisms by gently spreading the sediment out in a tray and removing the organisms using forceps or gently sieving the sediment.

After collection, rinse the organisms with clean seawater, blot them dry, and then weigh them. Measure the shell length of bivalves. Organisms should be analyzed immediately or frozen in baked-out aluminum foil or glass vials. The entire soft-tissue of each individual or composite of individuals from an experimental unit should be prepared for analysis. In many cases, the tissue from each experimental unit will first be homogenized and then subsamples taken for organic, metal, and lipid analyses, and archiving. The type of homogenization technique will depend upon size and tissue consistency of the organism, the pollutant of interest, and the analytical procedures used for pollutant analysis.

D. GUT PURGING

When a whole-body tissue analysis is conducted on a deposit-feeder, any pollutants associated with the mineral particles and detritus in the gut are included. Depending on the mass of sediment and the associated pollutant concentration, the gut sediment can measurably increase the apparent whole-body tissue residue. Allowing the organism to purge its gut (i.e., defecate) in uncontaminated sediment reduces or eliminates this positive bias.

TABLE X-1: Errors Associated With Gut Sediment/Purging

I. Gut Sediment Introduces Greatest Error:

1. In organisms that selectively ingest high organic particles.
2. In organisms with a large gut capacity.
3. During early stages of uptake when tissue residues are low.
4. For compounds not extensively bioaccumulated, especially high Koc compounds with steric hindrance to uptake.

II. Purging Introduces Greatest Error:

1. For rapidly depurated/metabolized compounds.
2. In organisms which do not clear gut in water.

However, pollutants will depurate or be metabolized during purging, resulting in an underestimation of the bioaccumulation. The type and extent of the error will depend upon many factors, including the feeding behavior of the organism and the nature of the pollutant. Factors influencing the errors associated with gut sediment or purging are summarized in Table X-1.

1. Standard 24-Hour Purge

Organic compounds with high Kow values (e.g., PCBs, DDT, BaP) are usually the greatest environmental concern in terms of bioaccumulation. Most of these compounds are slowly depurated, so a relatively small amount should be lost during purging. Therefore, we recommend a 24-hour gut purging as the standard procedure for sediments known or suspected to contain more than trace amounts of these pollutants. A 24-hour depuration period is sufficient for organisms to defecate the majority of their gut contents without introducing substantial errors from pollutant depuration or metabolism.

Many deposit-feeders require the ingestion of sediment to completely void their gut contents, so organisms should be placed in control sediment to assure complete purging. Reference sediment should not be used as the purging sediment. Maintain environmental conditions (e.g., temperature, salinity) as during the exposure phase. The organisms in the control and reference sediment(s) should undergo the same purging treatment as individuals exposed to the test sediment. Organisms from different treatments should be kept in separate containers to

avoid any possibility of cross-contamination. Observations should be made on whether feces were produced during the purging period and on the general health of the organisms.

There are several other techniques or modifications to the standard 24-hour purge which may be considered in specific cases. These methods are discussed in Appendix X-1.

2. When Not To Purge

There are certain situations when gut purging may introduce a greater error than leaving the gut sediment. In the first situation, the primary focus of the study is comparing laboratory and field studies. In most cases, it is impractical to purge field collected organisms. Therefore, to assure that the laboratory and field results are directly comparable, laboratory organisms should not be purged. In the second case, the primary focus is to determine the trophic transport of pollutants. As deposit-feeders extract sediment-associated pollutants in their guts (e.g., Lee et al., in press), it is likely that predators would also extract a certain percentage of the pollutants from their prey's gut sediment. In the final case, the primary focus is on lower molecular weight PAHs. These compounds can be depurated and metabolized rapidly (see Table X-2) so that a 24 hour purge can result in a greater error than leaving the gut sediment.

TABLE X-2: Depuration Loss Of Pollutants During 24 and 72 Hour Gut Purges

COMPOUND	ORGANISM	% LOST (HRS) 24	72	REF.
PCB	Crangon septemspinosa	3	8	McLeese et al., 1980
HCB	Macoma nasuta	4	12	unpublished data
BaP	Pontoporeia hoyi	4-28	11-64	Landrum & Poore, 1988
Phe	Pontoporeia hoyi	11-54	28-90	Landrum & Poore, 1988

PCB = Aroclor 1254
HCB = Hexachlorobenzene
BaP = Benzo(a)pyrene
Phe = Phenanthrene

E. ACCEPTABLE LEVELS OF MORTALITY

According to ASTM (1984) guidelines for bioconcentration tests, a test is unacceptable if "more than 10% of the organisms in any treatment died or showed signs of disease, stress, or other adverse effects." This criterion is applicable to studies of spiked sediments in which it is possible to adjust pollutant concentrations. Repeat any 28-day spiking experiment at a lower pollutant concentration if 10% or more of the organisms in any treatment die or show overt signs of stress. Signs of stress include avoidance of the sediment, non-burial, casting off of siphons, abnormal tube construction, and reduced ventilation or sediment processing rates.

In contrast to most experimental studies of bioavailability, many of the field sediments or dredge materials of environmental concern will have moderate to high toxicity. With these sediments, it may be impossible or difficult to meet the 10% mortality criterion. However, this may not represent a serious problem as the purpose of evaluating these sediments is to determine the extent of bioaccumulation which will result from a particular sediment. Presumably, the mortality in the laboratory would mimic the response in the field and so represent the actual effect of the sediment.

Because of the different purposes of tests conducted on field versus spiked sediments, we suggest not automatically rejecting bioassays with greater than 10% mortality in the test

sediment. The determining factor in deciding whether to accept a test treatment with high mortality is whether there are adequate replicates to obtain sufficient statistical power (see Chapter III). If the statistical power is insufficient, the experiment should be repeated. Also, mortality or stress in greater than 10% in the control or reference sediment would indicate initially stressed organisms, contamination of the system, or unacceptable control or reference sediment. In such cases, the cause of the problem should be determined and the experiment repeated. Consider using a more pollutant-resistant species or diluting the sediment to reduce toxicity (see Appendix V-1) in any future tests if the mortality in the test sediment exceeds 25%.

In some regards, high mortality in field sediments is a moot problem because any sediment sufficiently toxic to kill a substantial proportion of the recommended test species presumably would be unacceptable based on toxicity. However, even in cases where a sediment is rejected on the basis of toxicity, a bioaccumulation test conducted on the diluted sediment may help identify the compounds responsible for the toxicity.

F. CHAIN OF CUSTODY

In the event that litigation is expected, it is imperative to follow proper sample chain-of-custody procedures so that the results are acceptable for court. We recommend following the chain-of-custody procedures published by the National Enforcement

Investigations Center (U.S. EPA, 1986a). Other sources include "Quality Assurance and Quality Control (QA/QC) for 301(h) monitoring programs: Guidance on Field and Laboratory Methods" (Tetra Tech, 1986b) and U.S. EPA Contract Laboratory Program (U.S. EPA, 1988, 1989).

CHAPTER XI: POLLUTANT AND LIPID ANALYSIS

A. POLLUTANT ANALYSIS

The specifics of the techniques used to analyze sediment, water, and tissues for pollutants is a complex subject beyond the scope of this manual. Discussions of analytical techniques can be found in Tetra Tech (1985b, 1986f,g,h) and U.S. EPA (1988, 1989). It is possible, however, to offer several guidelines. First, analytical techniques are media dependent. Thus, time should be allocated for modifying the procedures for the various media and any special conditions (e.g., high TOC sediment, low tissue biomass). Second, a harsh extraction technique should not be used when analyzing sediments for metals since such a technique can extract biologically unavailable metals from the mineral matrix. A discussion of various metal extraction techniques is found in Luoma and Bryan (1978) and Waldichuk (1985). Third, to the extent possible, the PCB analysis should be at the level of identifying and reporting specific congeners rather than Aroclor equivalents. In particular, the more toxic planar congeners need to be identified. A thorough review of PCB congeners, including which to analyze, can be found in McFarland and Clarke (1989).

The required or desired detection limits will have a major effect on the choice of analytical techniques and on the ability to interpret the data. In some cases, the detection limits and analytical procedures will be specified by the pertinent regulation while in other cases the decision will be determined

by the researcher. If no detection limits are specified, we recommend that, at a minimum, the analytical techniques meet the requirements of the U.S. Environmental Protection Agency's Contract Laboratory Program requirements (U.S. EPA, 1988, 1989). The quantification limits from these documents are summarized in Table XI-1. These protocols cover both metals and organics in water and sediment. Although tissues are not covered by these protocols, it should be possible to obtain the same quantification limits as with the sediments.

Control samples or samples from relatively clean areas contain low concentrations of pollutants, and may require lower detection limits to achieve satisfactory results. The methods developed for measuring pollutants in samples collected from the PSDDA control sites in Puget Sound (U.S. ACE, 1988) are suggested in such cases. The PSDDA values include tissues as well as water and sediment, and are summarized in Table XI-2.

A complete quality assurance/quality control plan is a central part of any analytical procedure. Information on analytical QA/QC procedures are available from several sources (U.S. EPA, 1988, 1989; U.S. ACE, 1988). An important part of any QA/QC program is the use of reference samples and standards. Reference samples and standards are available from the U.S. EPA in Cincinnati, OH; Las Vegas, NV; and Research Triangle Park, NC, as well as the National Institute of Standards and Technology (Office of Standard Reference Materials, Room B311, Chemistry Building, NIST, Gaithersburg, MD 20899).

TABLE XI-1: U.S. EPA Contract Laboratory Program Quantitation Limits for Water and Sediment With Estimates for Tissue Matrices*

Organics	Water (a)	Sediment (b)	Tissue (c)
Volatiles	5-10	0.5-10	0.5-10
Semivolatiles	10-50	330-1600	330-1600
Pesticides/PCB's	0.05-1	8-160	8-160
For individual pollutants - refer to CLP Statement of Work			

Metals	Water (a)
Antimony	20-300
Arsenic	5-100
Cadmium	0.5-10
Copper	5-100
Lead	5-100
Mercury	0.2-20
Nickel	5-100
Silver	1-25
Zinc	0.2-4
Metals not listed - refer to CLP Statement of Work	

a = ug/L
b = ug/kg wet weight
c = ug/kg wet weight basis. These values were estimated from the sediment values on the premise that tissue and sediment pollutant concentrations are of a similar magnitude and are analyzed by similar techniques.

TABLE XI-2. PSDDA Low Limits of Detection for Water, Sediment and Tissue Matrices

Organics	Sediment (a)	Tissue (b)
Volatiles	10-20	5-10
Semivolatiles	1-50	10-20
Pesticides/PCB's	0.1-15	0.1-20

Metals	Water (c)	Sediment (d)	Tissue (e)
Antimony	3	0.1	0.02
Arsenic	1	0.1	0.02
Cadmium	0.1	0.1	0.01
Copper	1	0.1	0.01
Lead	1	0.1	0.03
Mercury	0.2	0.01	0.01
Nickel	1	0.1	0.02
Silver	0.2	0.1	0.01
Zinc	1	0.2	0.20

a = ug/kg dry weight (ppb)
b = ug/kg wet weight (ppb)
c = ug/L (ppb)
d = mg/kg dry weight (ppm)
e = mg/kg wet weight (ppm)

B. LIPID ANALYSIS

A number of studies have demonstrated that lipids are the major storage site for organic pollutants in a variety of organisms (Roberts et al., 1977; Oliver and Niimi, 1983; de Boer, 1988). Because of the importance of lipids, bioaccumulation programs have recently attempted to normalize tissue pollutant concentrations to the tissue lipid concentration. For example, lipid concentration is one of the factors required in deriving the Accumulation Factor (AF) (see Appendix I-1). The approach, however, has experienced difficulties because of the differences in the lipid concentrations reported from the wealth of different lipid methods used (see Kates, 1986 for discussion of lipid methodology). Work in this laboratory has shown that differences in lipid technique can result in 3-fold differences in lipid concentrations. These differences in lipid concentrations directly translate into a similar variation in the lipid normalized pollutant concentrations or Accumulation Factors.

To allow lipid normalized tissue residues or AFs to be compared, it is necessary to either promulgate a standard lipid technique or to intercalibrate the various techniques. Standardization on a single method is difficult because the lipid methodology is often intimately tied in with the extraction procedure for pollutant analysis. Instead, we recommend that one lipid technique be chosen as an "intercalibration standard". Then, regardless of what method is used, the results would be reported in equivalent units of the standard.

The difficulty with this approach is deciding upon which technique to use as the standard. We are presently investigating the advantages and disadvantages of different techniques, but have not yet reached a consensus. As in interim solution, we suggest the Bligh-Dyer lipid method (Bligh and Dyer, 1959) as a temporary "intercalibration standard". Folch et al. (1957) developed a total lipid method that extracts the neutral and polar lipids (i.e. total lipids) from biological samples using chloroform and methanol as the solvent system. Bligh and Dyer (1959) improved upon the method by providing a cleanup for the extracted lipid residues.

The potential advantages of Bligh-Dyer include its ability to extract neutral lipids not extracted by many other solvent systems and the use of Bligh-Dyer (or the same solvent system) in numerous biological and toxicological studies (e.g., Roberts et al., 1977; Oliver and Nimi, 1983; de Boer, 1988; Landrum, 1989). Because the technique is independent of any particular analytical extraction procedure, it will not change when the extraction technique is modified or changed. Additionally, the method can be modified for small tissue sample sizes as long as the solvent ratios are maintained. We have successfully used a modified technique with tissue samples as small as 1 g wet tissue and micromethods using chloroform - methanol requiring only milligram amounts of tissue were developed by Herbes and Allen (1983) and Gardner et al. (1985).

One potential disadvantage of the Bligh-Dyer is that by extracting many of the lipids not extracted by other techniques, it may be extracting lipids that are not important to the storage of neutral organic pollutants. Seasonal changes or interspecific variations in these non-active lipid fraction could obscure the relationship between lipid content and pollutant accumulation. However, the standard Bligh-Dyer technique may not solublize triglycerides (Gagney, P., pers. comm., U.S. EPA, ERL-A, Athens, GA), thereby underestimating the lipid pool important to pollutant storage. Other drawbacks are the use of chloroform, which is a carcinogen, and the need to conduct an additional analysis instead of measuring the lipids as part of the normal organic extraction procedure (e.g., Rubinstein et al., 1987; Lake et al., in review). However, the alternative lipid methods all have similar limitations, and we believe that Bligh-Dyer is the best interim calibration method.

If the Bligh-Dyer method is not used as the primary lipid method, compare the chosen lipid method with Bligh-Dyer for each tissue type. The chosen lipid method could then be converted to "Bligh-Dyer" equivalents and the lipid normalized tissue residues could then be reported in "Bligh-Dyer equivalents". Because of the interim nature of this suggestion, we also suggest that extra tissue of each species be frozen for future lipid analysis in the event that a different technique proves more advantageous.

C. SAMPLE STORAGE

For organics, the U.S. EPA Contract Laboratory Program (U.S. EPA, 1988) requires that the samples be protected from light and refrigerated at 4°C (± 2°C.) from the time of receipt until they are extracted and analyzed. Water samples shall be extracted within 5 days of the receipt of the sample. Sediment samples shall be extracted within 10 days of the receipt of the sample and if continuous extraction procedures are employed, extraction of water samples shall be started within 5 days of the receipt of the sample.

For inorganics, the U.S. EPA Contract Laboratory Program (U.S. EPA, 1989) requires that soil and sediment samples be maintained at 4°C. (± 2°C.) until analyzed. Samples for mercury shall be analyzed within 26 days of the receipt of the sample. Samples for metals shall be analyzed within 180 days of the receipt of the sample.

At times, other program priorities (e.g., analysis of archived samples) do not allow one to abide by the requirements set by the Contract Laboratory Program. In those cases, it is suggested that the samples either be frozen (-20°C) in air tight containers or dried depending on the type of sample and the analyses required. Purging the container with nitrogen prior to sealing will delay the degradation of some pollutants as well as lipids. Sample containers should be as full as practical to prevent loss of moisture from the sample. Sediment samples so

preserved are stable for at least 6 months, if not longer (Tetra Tech, 1986d). Tissue and water samples are expected to be at least as stable as sediments.

D. REPORTING OF RESULTS

Investigators have reported results on either a dry or wet basis, usually without a conversion factor between the two and sometimes without any indication of which was used. This makes it difficult, or impossible, to compare results from different studies. In general, a dry-weight basis is preferred for both sediment and tissue pollutant concentrations. However, certain analytical techniques use wet tissue or wet sediment, necessitating the calculation of wet-weight concentrations. To allow comparisons among studies, the wet-to-dry weight ratios should be reported for each tissue and sediment type. As mentioned above, lipid values should be reported in "Bligh-Dyer equivalents" along with any conversion factor(s) between lipid methods.

CHAPTER XII: STATISTICAL ANALYSES

The main objective of statistical testing is to determine whether the mean tissue residues in animals exposed to the test sediment are significantly greater from those in the control and/or reference sediments, or greater than a specified criterion value such as an FDA Action Limit. Additional statistical tests comparing the means of other tissue residues (e.g., control vs reference) or sediment characteristics will also be conducted, but the same principles and methods apply. A summary of the standard statistical tests and their interpretation are summarized in Table XII-1 and Table XII-2.

To perform statistical testing, replicate samples must have been taken to provide an estimate of variability. Non-replicated samples (i.e. concentration from a single composite sample) cannot be compared using these methods. In these tests, the concentration of each chemical in a tissue or sediment sample is considered statistically independent and is compared separately. Comparisons of tissue residues of different chemicals within the same organisms requires the use of "repeated measures" (Section E).

Standard deviations (SD or s) or standard errors (SE) and number of the replicates (n) should always be reported in addition to mean values. When composited values are used, report the number of organisms per composite (if the composite comprises the experimental unit) or the number of experimental units per composite, as well as the number of replicate composites sampled.

Prior to conducting any statistical analyses, it is necessary to decide whether the comparisons between means are to be multiple or pairwise. Pairwise comparisons include comparisons of a test and control/reference mean for tissue concentrations, sediment characteristics, etc. Pairwise comparisons also include the comparison of the control with the reference mean and comparisons of a mean and a specified criterion value such as comparison of a test tissue residue with an FDA action limit. Multiple comparisons involve comparisons of more than two means simultaneously. Multiple comparisons are used in cases such as determining whether three or more test tissue concentration means are equal or whether all the TOC values for the sediments (test(s), control and reference) are equal.

After the applicable comparisons are determined, the data need to be tested for normality to determine whether parametric statistics are appropriate and whether the variances of the means to be compared are homogeneous. If normality and homogeneity of variances are established, t-tests can be performed in the case of pairwise comparisons or ANOVA in the case of multiple comparisons.

A. TESTS FOR NORMALITY AND HOMOGENEITY OF VARIANCES

Before conducting parametric statistics, the data need to be checked for both normality and homogeneity of variances. The data for each chemical or sediment parameter are tested separately. Commonly used tests for testing normality are the

Kolmogorov-Smirnov one-sample test and the chi-square test (Sokal and Rohlf, 1981). However, these tests are not very powerful, especially if sample sizes are small (such as 8 replicates). More powerful, but less common, tests of normality such as Shapiro-Wilk and K^2 tests (D'Agostino and Stephens, 1986) can be used for small sample sizes.

If the data are not normally distributed, the data can often can be transformed to achieve normality. The logarithmic and arcsine are two commonly used transformations for concentrations. It may be necessary to apply different transformations to different chemical or sediment parameters. See Sokal and Rohlf (1981) for a more extensive discussion on transformations. If normality cannot be established, nonparametric tests for comparisons of two means, such as the Mann-Whitney test and the Tukey's Quick test should be used. These non-parametric tests are usually not as powerful as the more common parametric tests, such as the t-test or Analysis of Variance (ANOVA). See Daniel (1978) for a discussion of non-parametric statistics.

The variances of the samples to be compared should be tested for homogeneity. This is performed using an F-test when comparing two variances or Bartlett's test when comparing more than two variances. If the variances are considered homogeneous, then a t-test or ANOVA is appropriate. If the variances are heterogeneous, the data can be transformed in an attempt to achieve homogeneity. Under conditions of variance heterogeneity, a modified t-test for comparisons of two means or

approximate tests for multiple comparisons can be performed. See Sokal and Rohlf (1981) for a more extensive discussion on appropriate tests when different treatments have unequal variances.

B. PAIRWISE COMPARISONS

Pairwise comparisons are performed using Student's t-test, using a pooled variance estimate when variances are homogeneous. Under conditions of variance heterogeneity, a modified t-test can be used (see Sokol and Rohlf, 1981). Prior to analysis, it must be established whether the t-test performed will be a one-tailed or two-tailed test and whether the Type I error rate should be a comparison-wise or experiment-wise error rate. These considerations are discussed below.

1. One-Tailed versus Two-Tailed Tests

In formulating a statistical hypothesis, the alternative hypothesis can be one-sided (one-tailed test) or two-sided (two-tailed test). The null hypothesis (Ho) is always whether two values are equal. A one-sided alternative hypothesis (Ha) is that there is a specified relationship between the two values (e.g., one value is greater than the other) versus a two-sided alternative hypothesis (Ha) which is that the two values are simply different. A one-tailed test is used when there is an a priori reason to test for a specific relationship between two means such as the alternative hypothesis that the test tissue residue is greater than the control tissue residue. In contrast,

the two-tailed test is used when the direction of the difference is not important or cannot be assumed prior to testing. An example of an alternative two-sided hypothesis is that the reference sediment TOC is simply different from the control sediment TOC.

Because control tissue residues and sediment pollutant concentrations are presumed lower than reference values which are presumed lower than test values, we recommend conducting one-tailed tests in most cases. For the same number of replicates, one-tailed tests are more likely to detect statistically significant differences between treatments (i.e., have a greater power). This is a critical consideration when dealing with a small number of replicates (such as 8 per treatment). The other alternative to increasing statistical power is to increase the number of replicates, which increases the cost of the bioassay.

The use of one-tailed tests deviates from the usual experimental procedure, but is justified where a regulatory action would be taken only if the tissue residues in organisms exposed to a test sediment were greater than those in a control or reference sediment. For example, a dredge material might be denied disposal in open water if the tissue residues in the test sediment (i.e., dredge material) were significantly greater than those in the reference sediment. However, the same regulatory decision (i.e., allow disposal) would be reached whether the tissue residues in the test sediment were equal to or less than those in the reference sediment. The same reasoning would apply when comparing a tissue residue to a tissue criterion.

There are cases when a one-tailed test is inappropriate. When no a priori assumption can be made as to which treatment is higher than the other, a two-tailed test should be used. For example, when comparing TOCs of the test and reference sediments, a two-tailed test should be used. A two-tailed test should also be used when one regulatory action will be taken when the two treatments are equal and another when they are not equal, regardless of which one was larger or smaller. This would be unusual for tissue residues, but would apply to other benthic parameters. For example, a two-tailed test should be used when comparing benthic biomass at a control and test site because both enhanced and reduced biomass are indicators of organic enrichment (Pearson and Rosenberg, 1978), so the regulatory question is whether there is any difference between the two sites. A two-tailed test should also be used when comparing tissue residues among different species exposed to the same sediment and when comparing BAFs or AFs (see Appendix I-1).

The appropriate one-tailed and two-tailed tests for the bioaccumulation test are summarized in Table XII-1 and Table XII-2.

2. Comparison-wise versus Experiment-wise Error Rates

The Type I error rate used in the tests will be chosen either as a comparison-wise or experiment-wise error rate depending on whether one decision is made for each pairwise comparison or from a set of pairwise comparisons. For cases where test sediments are chosen in a stratified manner or along a

TABLE XII-1: Summary of Statistical Analyses

PAIRWISE COMPARISONS

Hypothesis	Test(s)*	Comments
Normality	Chi-square or Kolmogorov-Smirnov	Try transformations if not normal
Equality of Variances	F-test	Try transformations if not equal
Equality of Means	t-test	One-tailed with a priori knowledge otherwise two-tailed
Equality of Means	modified t-test	If variances are not equal
Equality of a Mean and a constant	t-test	One-tailed with a priori knowledge otherwise two-tailed
Equality of Means	nonparametric tests	If normality is not established

MULTIPLE COMPARISONS

Hypothesis	Test(s)*	Comments
Normality	Chi-square or Kolmogorov-Smirnov	Try transformations if not normal
Equality of Variances	Bartlett's test	Try transformations if not equal
Equality of Means	ANOVA	If normality is established
Equality of Means	nonparametric tests	If normality is not established

* Often more than one test can be used for the same hypothesis. Each test will have different assumptions. Chose the test with the assumptions most closely matching your specific conditions and requirements.

gradient (see examples a and b in Figure XII-1) and any decisions will be made on a case by case basis, a comparison-wise Type I error rate of 0.05 should be used for each comparison. For example, a comparison-wise error would be used in deciding which specific stations along a gradient were acceptable or not acceptable.

If the test sediments are selected from a supposedly homogeneous source (e.g., multiple sediment samples from a dredge barge, see example c in Figure XII-1) and the decision to accept or reject the sediment will be made from the results of several pairwise comparisons, then an experiment-wise error rate of 0.05 should be used. In this example, a regulatory decision will depend on the results from all the comparisons of the test treatments to determine if the sediment in the barge is too contaminated for disposal. Each individual comparison is performed at a lower error rate such that the probability of making a Type I error in the entire series of comparisons is not greater than 0.05. This results in a more conservative test when comparing any particular sample to the control/reference. Thus, a single sediment sample from the barge that would have been rejected at the 0.05 level may not be rejected at the lower experiment-wise error rate, though the probability of rejecting Ho for the entire set of samples is still 0.05. Use of experiment-wise error rates adjusts for the possibility of random differences when multiple samples are taken from a homogeneous source (e.g., if 100 samples were taken, a certain percentage

would be greater than the control/reference because of random fluctuations). The error rate used in each comparison is a function of the number of comparisons to be used in the decision "experiment" and can be computed using the method of Dunn-Sidak (Sokol and Rohlf, 1981) as:

$$alpha' = 1-(1-alpha)^{1/k} \qquad (1)$$

where:

alpha' = Type I error rate used for each pairwise comparison

alpha = experiment-wise Type I error rate (0.05)

k = number of comparisons

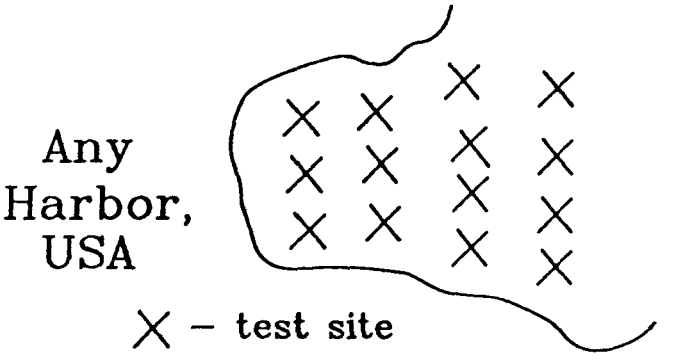
When an experiment-wise error is used, the power to detect real differences between any two means decreases as a function of k, the number of comparisons.

C. MULTIPLE COMPARISONS

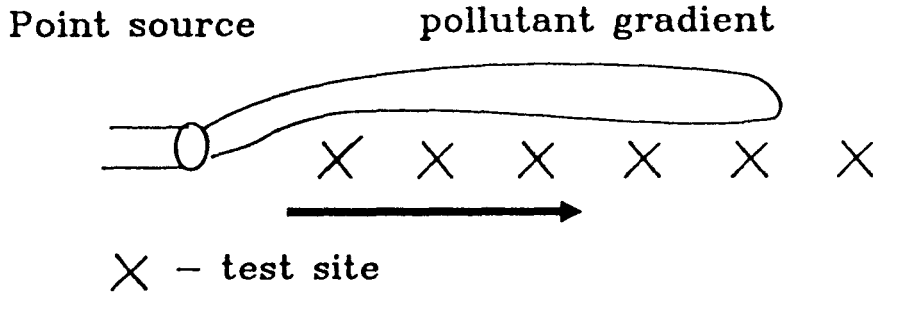
For comparisons involving several means, as in the case of comparing TOC values among all sediment types, an ANOVA is first performed to establish whether any of the means are different. The ANOVA also provides a "best" estimate of the variance (within-treatment error). If there are significant differences, a series of t-tests can be performed for any planned (a priori) comparisons (such as between test and control/reference) to distinguish which means are different. For unplanned (a posteriori) comparisons, such as between two reference tissue

FIGURE XII–1
Sampling Schemes
for Comparison–wise (a. and b.)
vs Experiment–wise (c.) Error rates

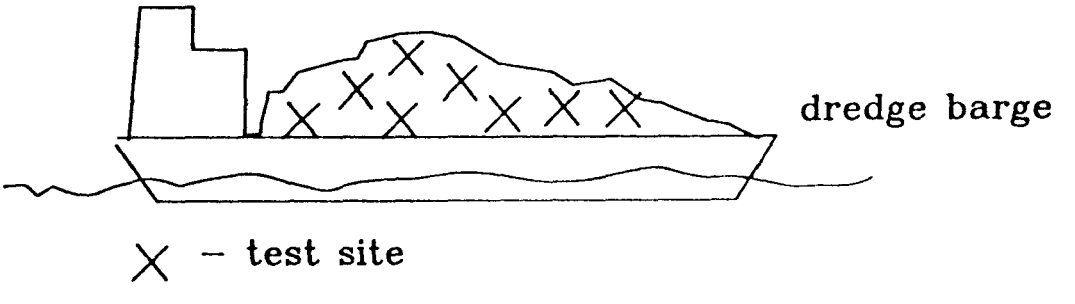
a. Stratified selection of test sediments



b. Selection of test sediments along a gradient



c. Selection of test sediments from a presumably homogenous source



residues, tests such as the T-Method or Tukey Kramer procedure (Dunnett's test) are more appropriate. See Sokal and Rohlf (1981) for unplanned multiple comparison tests to determine which is most suited for each case.

It is important to note that an ANOVA is inherently for two-tailed comparisons. Therefore if the comparisons can be broken down into a series of one-tailed pairwise comparisons, it is preferable to perform the analysis in this manner because of the increase in power. However, if the series of comparisons are two-tailed, an ANOVA can be performed first to determine whether any additional comparisons should be made.

D. INTERPRETATION OF COMPARISONS OF TISSUE RESIDUES

If the control mean tissue residues at day 28 are not significantly greater than the day 0 tissue residues, it can be concluded that there is no significant contamination from the exposure system or from the control sediment. If there is significant uptake, the exposure system and/or control sediment should be reevaluated as to suitability. Even if there is a significant uptake in the controls, it is still possible to compare the controls and treatments as long as the pollutant concentrations in the test tissue residues are substantially higher. However, if control values are high, the data should be discarded and the experiment conducted again after determining the source of contamination.

Comparisons between the 28-day control (and/or reference) tissue residues and 28-day test tissue residues determines whether there is statistically significant bioaccumulation due to exposure to test sediment. Comparisons between control and reference tissue residues at day 28 determines whether there is statistically significant bioaccumulation due to exposure to the reference sediment. When test tissue residues are compared with a one-tailed test with a set criterion value (e.g., FDA Action Limit), if no significant difference is detected, the residues must be considered equivalent to the value even though numerically the mean tissue residue may be lower.

The statistical interpretation of these and other tests are summarized in Table XII-2.

E. ADDITIONAL ANALYSES

1. Testing BAFs and AFs

Statistical comparisons between ratios such as BAFs or AFs are difficult due to computation of error terms. Since all variables used to compute BAFs and AFs have errors associated with them, it is necessary to estimate the variance as a function of these errors. This can be accomplished using approximation techniques such as the propagation of error (Beers, 1957) or a Taylor series expansion method (Mood et al., 1974). BAFs and AFs can then be compared using these estimates for the variance. See Ferraro et al. (1990) for an example of this approach.

TABLE XII-2: EXAMPLES OF ANALYSES AND INTERPRETATION OF RESULTS

HYPOTHESIS	TEST	INTERPRETATION OF REJECTION OF NULL HYPOTHESIS
PHYSICAL PARAMETERS		
Ho: $TOC_c=TOC_i$ Ha: $TOC_c \neq TOC_i$	two-tailed t-test	TOC not equal between control and test sediment i
Ho: $TOC_c=TOC_r$ Ha: $TOC_c \neq TOC_r$	two-tailed t-test	TOC not equal between control and reference
Ho: $TOC_c=TOC_r=TOC_1=...=TOC_n$ Ha: $TOC_c \neq TOC_r \neq TOC_1=... \neq TOC_n$	ANOVA	TOC of one or more sediment differs
ADEQUACY OF CONTROL		
Ho: $Ct_c=Ct_u$ Ha: $Ct_c>Ct_u$	one-tailed t-test	Exposure system contaminated
TREATMENT DIFFERENCES		
Ho: $Ct_i=Ct_c$ Ha: $Ct_i>Ct_c$	one-tailed t-test	Sig. uptake from test sediment i above control
Ho: $Ct_i=Ct_r$ Ha: $Ct_i>Ct_r$	one-tailed t-test	Sig. uptake from test sediment i above reference
Ho: $Ct_r=Ct_c$ Ha: $Ct_r>Ct_c$	one-tailed t-test	Sig. uptake from reference sediment above control
Ho: $Ct_c=Ct_r=Ct_1=...=Ct_n$ Ha: $Ct_c \neq Ct_r \neq Ct_1=... \neq Ct_n$	ANOVA	Uptake from one or more sediment differs
Ho: $Ct_1=Ct_2=...=Ct_n$ Ha: $Ct_1 \neq Ct_2=... \neq Ct_n$	ANOVA	Uptake from one or more test sediment differs
LONG TERM EXPOSURES		
Ho: $Ct(j)_i=Ct(j+1)_i=Ct(j+3)_i$ Ha: $Ct(j)_i \neq Ct(j-1)_i \neq Ct(j-3)_i$	ANOVA	Ct_i has not reached steady-state

Ho = null hypothesis
Ha = alternative hypothesis
Ct = concentration of pollutant in tissue at day 28
Subscripts: c = control organisms or sediment
i = 1,2,..,n test organisms or sediment
j = last sampling period
n = total number of test treatments
r = reference organisms or sediment
u = unexposed organisms

2. Comparing Tissue Residues of Different Compounds

In some cases, it is of interest to compare the tissue residues of different compounds. For example, Rubinstein et al. (1987) compared the uptake of thirteen different PCB congeners to test for differences in bioavailability. Because the values for the different compounds are derived from the same tissue samples, they are not independent and tend to be correlated, so standard t-tests and ANOVAs are inappropriate. Rather, a repeated measures technique (repeated testing of the same individual) should be used where the individual (experimental unit) is considered as a random factor and the different compounds as a second factor. See Rubinstein et al. (1987) and Lake et al. (in review) for an example of the application of repeated measures to bioaccumulation data.

3. Analyses for Alternative Test Designs

Long-term exposures require a test to show that steady-state has been reached. An ANOVA should be performed on the last three sample sets. ASTM (1984) requires that there be no significant difference ($p>.05$) between the means of these sample sets. If apparent steady-state is reached, the mean of the samples taken during apparent steady-state should be used for the steady-state concentration value. For steady-state estimates based on uptake and depuration tests, see Davies and Dobbs (1984) or Spacie and Hamelink (1982) for details on the nonlinear parameter estimation methods required to estimate these rate constants and steady-state concentrations.

CHAPTER XIII: REGULATORY STRATEGIES FOR USE OF BIOACCUMULATION DATA

Bioaccumulation is the link between exposure and effects, and thus can generate important insights into ecological effects, human health risks, and the routes and extent of pollutant exposure. However, its use in a regulatory context is not as straightforward as acute toxicity data. Death is unequivocal and undeniably bad for an individual organism. But what of a PCB tissue residue of 1.5 ppm, or one of 50 ppb? The answer to questions such as this are often not clear. Yet, many of today's environmental problems are due to the accumulation and trophic transport of sublethal concentration of pollutants rather than major die-offs. Bioaccumulation and consumption of contaminated seafood is certainly one of the primary environmental concerns of the public.

In the previous chapter (Chapter XII), the statistical procedures to test for increases in tissue residues were discussed. It is important to note that statistical differences in themselves do not necessarily indicate an environmental or human health problem. Conversely, the lack of statistically greater tissue residues in test sediment compared to a proper control would be strong evidence that the test sediment would not result in an environmental or human health problem for the pollutants tested. The lack of a statistical difference between a test and reference sediment would indicate that the environmental problems resulting from the test sediment would be

no worse than those from the existing reference sediment. Whether this is environmentally acceptable depends on the present environmental conditions at the reference site.

In this Chapter, we summarize some of the approaches to interpreting the ecological and human health ramifications of a significant increase in tissue residues in a test sediment or dredge material. Some of the approaches are well established, while other are still in the conceptualization stage. We only consider bioaccumulation data in this Chapter, whereas an environmental assessment would normally include sediment toxicity testing, benthic community analysis, effluent testing, or a number of other approaches. These various approaches generate different types of information which complement each other.

A. NO FURTHER DEGRADATION

1. Approach

Compare tissue residues in organisms exposed to a test sediment to those exposed to an appropriate reference sediment. If the test tissue residues are not greater than those in the reference sediment, it is concluded that the test sediment would not result in a degradation of existing environmental conditions.

2. Advantages and Applicability

The approach is straightforward and does not require any data other than the bioaccumulation tests. Comparison of existing or predicted pollution effects to present ecological

conditions is an established regulatory approach (e.g., 301(h)). This approach should be applicable in any area in which an appropriate reference sediment can be found.

3. Disadvantages

One potential problem with any approach that uses a field comparison is the choice of an appropriate reference site. As discussed in Chapter II, it is possible to get no statistical difference between test and reference tissue residues, but still result in unacceptable degradation. Without national guidelines, there may be large differences in the "environmental quality" of reference sites, and hence, in allowable tissue residues.

B: TISSUE RESIDUE EFFECTS

1. Approach

Relate the tissue residues to specific physiological or biochemical effects on the organism.

2. Advantages and Applicability

The advantage of this approach is that it relates the tissue residues to an effect on an organism. If a biochemical end-point or "biomarker" is used, the approach can generate insights into the mechanisms of stress. If such relationships can be developed, it would be a relatively simple matter to assess the environmental quality from tissue residues. The approach is applicable to all organisms, and depending on the end-points used, can be adapted for different sensitivities (e.g., early warning vs. unacceptable impacts).

3. Disadvantages

Because of the various mechanisms by which different pollutants can affect organisms, and because the actual mechanisms of toxicity may vary among taxa, there is often a weak correlation among tissue residues and effects. The relationship may also be weak if the stress is due to a small fraction of the tissue residue (e.g., pollutant affect on nerves). Presently, most of the techniques are not simple and often require expensive equipment.

C. WATER QUALITY CRITERION TISSUE LEVEL APPROACH

1. Approach

Tissue residues are compared to those residues that would occur at a water exposure to the Water Quality Criterion (WQC) concentration. The tissue concentration which would result from exposure to pollutants at the WQC are calculated by multiplying the BCF for a compound by the WQC. This tissue concentration is the "Water Quality Criterion Tissue Level" (WQCTL). Tissue residues higher than the WQCTL indicate that the integrated exposure through all routes was greater than allowed under WQC, and thus is unacceptable. This approach is similar to the Equilibrium Partitioning approach to deriving Sediment Quality Criteria in its use of the WQC as the end-points, except that tissue residues are used as the measure of exposure rather than interstitial water concentrations.

2. Advantages and Applicability

As with the Equilibrium Partitioning Approach, the WQCTL approach is relatively simple, draws upon extensive previous toxicological work, and is based on a well established regulatory approach (WQC). Furthermore, using tissue residues as a measure of the bioavailable pollutant concentration avoids the numerous factors which can affect the concentrations and bioavailability of interstitial water pollutants (e.g., complexation with DOM, the "solids effects", sampling artifacts, etc.). Also, this approach does not assume that interstitial water is the only uptake route or that an organism's tissue residues can not exceed a thermodynamic maximum. As both of these assumptions appear to be incorrect for some species (e.g., selective deposit-feeders) with some compounds (e.g., HCB, BaP), relaxing these assumptions increases the applicability of using WQC as the basis for regulating sediments. This approach could be used with any compound that had a chronic WQC value.

3. Disadvantages

This is a new proposal, and the idea has yet to be evaluated. As with the Equilibrium Partitioning approach, it assumes that WQC values are applicable to benthic organisms. The consistency of BCFs among water column organisms and BAFs or AFs among benthic species needs to be evaluated. With benthic species, the actual exposure may depend upon feeding type (Lee et al., in press) or source of ventilated water (Winsor et al., in press). Therefore, the exposure calculated using one species may

not be applicable to a different species. Rapidly metabolized compounds may give variable results. Finally, to the extent that the WQC was based on tissue residues, the approach may be circular.

D. FDA ACTION LIMITS

1. Approach

Compare the tissue residues in the benthic test species with FDA Action Limits. Tissue residues exceeding an FDA Action Limit are considered unacceptable.

2. Advantages and Applicability

The FDA Action Limits are a well established regulatory criteria. The FDA Action Limits are used as end-points in the evaluation of dredge materials (U.S. EPA/U.S. ACE, 1988).

3. Disadvantages

There are only a few FDA action limits for seafood. There is concern that the criteria are not sufficiently protective of human health, especially with high seafood consumption rates. The Action Limits do not consider ecological impacts. Because of the human health and ecological limitations, FDA Action Limits can be considered "one sided" criteria, where exceeding the limits is unacceptable but failure to exceed the limits is not strong evidence for acceptability of a sediment.

E. HUMAN HEALTH RISK ASSOCIATED WITH SHELLFISH

1. Approach

Calculate the excess human cancer risk from the consumption of contaminated shellfish. The calculation of cancer risk requires an estimate of the tissue residues, a cancer potency value, and a lifetime consumption rate of the shellfish. Non-cancer human health risks could also be calculated, though in most cases, cancer will be the greater health risk. This approach differs from using FDA Action Limits in that the risk is based on an estimate of actual human exposure. Therefore, the actual allowable concentration would depend upon the rates and patterns of the consumption of shellfish in an area.

2. Advantages and Applicability

Determining the excess cancer risk associated with consumption of seafood is of obvious and direct concern to the public. Such an approach would be applicable in all areas where shellfish are harvested recreationally or commercially. The human cancer model is an established regulatory method. Cancer potency values are available for many of the environmentally relevant pollutants. For some compounds (e.g., PCBs, BaP), human health risk can generate a lower acceptable sediment concentration than sediment toxicity (Lee and Randall, 1988), so for these compounds, this approach is protective of the environment and human health.

3. Disadvantages.

The local per capita consumption rates of shellfish is poorly known. Policy decisions must be made about what constitutes an acceptable human health risk (e.g., 10^{-5} versus 10^{-6} excess cancer risks) and whether to regulate on an average consumption or the maximum consumption rates (e.g., subsistence fishing by certain ethnic groups). Use of default seafood consumption rates for a site can generate unrealistically low safe sediment concentrations in areas with little or no harvesting of shellfish.

F. TROPHIC TRANSFER OF POLLUTANTS INTO PELAGIC FOOD WEBS

1. Approach

Predict the movement of pollutants from the benthos into their predators and through the food web, up to and including human consumers. The acceptability of a benthic tissue concentration would be determined from the human health effects associated with consumption of contaminated fishes (or ecological effects, if end-points were available). The same human cancer risk models would be used as used in calculating the risk associated with shellfish. The use of human health risk models with seafood is discussed in Tetra Tech (1986e). This Trophic Transfer approach is a generalized form of the Shellfish approach and the two would normally be combined (see Figure XIII-1).

2. Advantages and Applicability

Trophic transfer of contaminants from the benthos to their predators is one of the major mechanisms by which certain

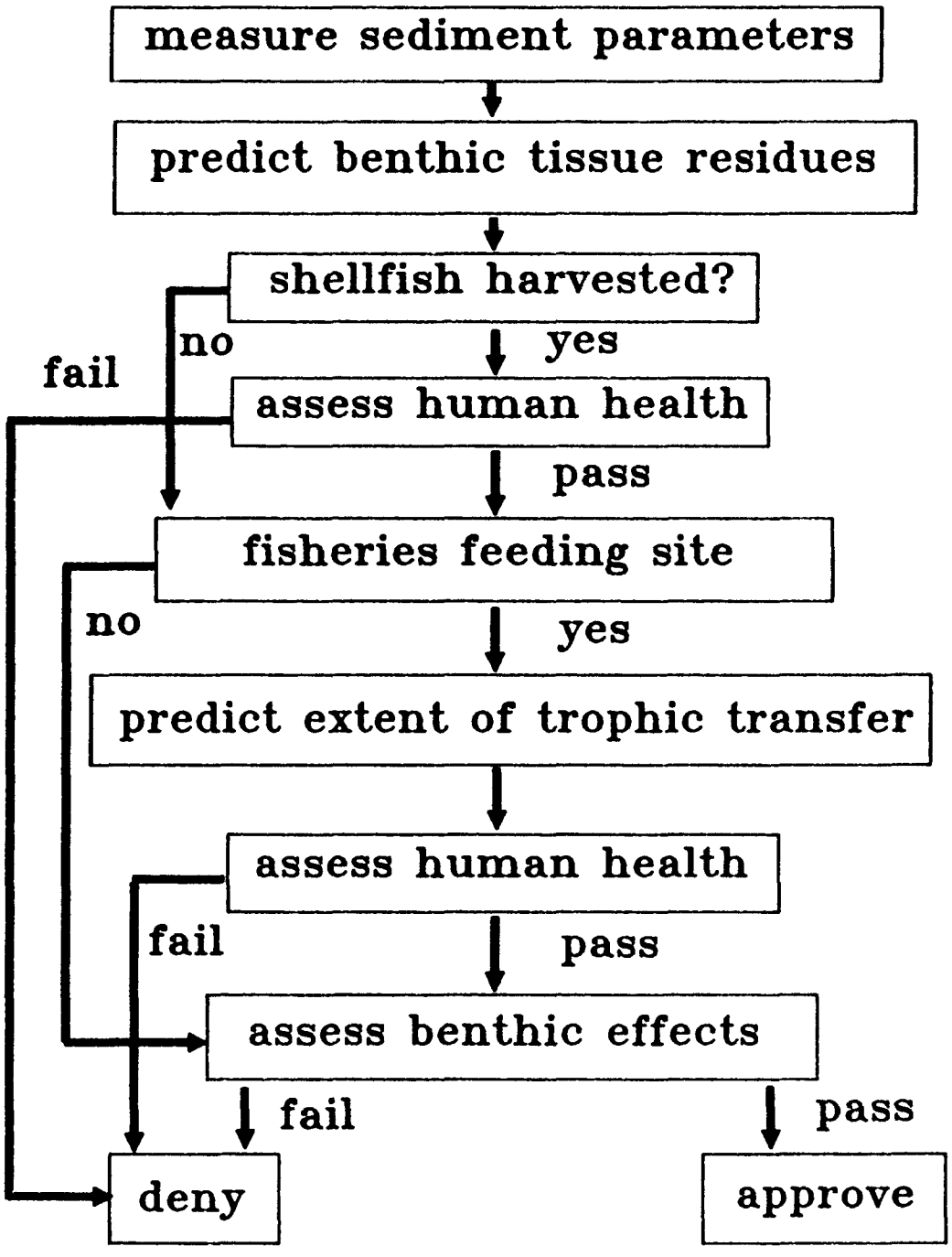
pollutants are introduced into pelagic food webs. This approach addresses the far-field effects of a contaminant as well as the near-field effects. The approach would be applicable in any area where fish are harvested recreationally or commercially. Models predicting the average increases in various pollutants per trophic level have been developed (Young et al., 1987; Young, 1988).

3. Disadvantages

One limitation of this approach is the difficulty in obtaining the data required to quantify the transfer of pollutants from the benthos to their predators and through the food web. The other major limitation is the uncertainty in assigning human seafood consumption rates.

FIGURE XIII–1

POSSIBLE REGULATORY STRATEGY FOR HUMAN HEALTH CRITERIA IN ASSESSING SEDIMENT CONTAMINATION



APPENDIX I-1: ADDITIONAL METHODS TO PREDICT BIOACCUMULATION

A. FIELD COLLECTION

The most direct method of assessing tissue residues in existing sediments is to measure residues in field collected organisms. The field approach is appealing because it avoids any laboratory artifacts, as well as the time, expense and facilities required for laboratory tests. However, use of field collected organisms has several limitations as a routine method.

The greatest problem is collecting sufficient tissue biomass of an appropriate species for chemical analysis. This problem is especially acute at the most contaminated sites because smaller species tend to dominate stressed communities and during the early stages of recolonization (Pearson and Rosenberg, 1978; Rhoads et al., 1978). In addition, benthic densities are reduced under severe stress (Pearson and Rosenberg, 1978). Even when sufficient biomass of a particular species can be collected at a given station, it will often be impossible to collect the same species from other stations located along a pollution gradient, seasonally within a single station, or at an estuarine dredge site and an open ocean disposal site.

One possible approach to collecting sufficient biomass is to composite the various species collected from each site. Although mixing species will increase biomass, tissue composites taken from different stations or seasons are likely to be composed of substantially different proportions of species and numbers of individuals. These compounding factors will make it unclear

whether patterns in tissue residues are due to differences in the sites or interspecific differences in bioaccumulation. For example, amphipods have a much greater ability to metabolize PAHs compared to bivalves (Varanasi et al., 1985). Therefore, a difference in PAH tissue residues among sites could reflect a difference in the proportion of amphipods and bivalves rather than a difference in the bioavailability of PAHs.

Another problem is that the exposure history of field collected specimens is usually unknown. Many benthic species, especially amphipods and some polychaetes, are mobile during a portion of their life-history (e.g., Williams and Porter, 1971; Williams and Bynum, 1972; DeWitt, 1988) and may have migrated into a site recently. Although pollutant concentrations in sediments are usually considered relatively constant, resuspension events can obscure sediment-bioaccumulation relationships. For example, deposition of resuspended contaminated sediments in an uncontaminated site would form a surface veneer available to surface-deposit feeders or filter-feeders. In this were the case, a bulk sediment analysis would underestimate the actual exposure. Also, field organisms are potentially exposed to contaminated phytoplankton and to pollutants dissolved in the overlying water. If these water column routes are important, then relating tissue residues to the field sediment would generate incorrect conclusions regarding sediment bioavailability.

With these limitations, field collections are not as well suited as laboratory experiments for the routine prediction of the tissue residues resulting from dredge materials and pollutant discharges, or for between-site comparisons of sediment bioavailability. Field collections are, however, a powerful regulatory tool if used in the context of periodic monitoring of existing sites. In comparing changes at the same stations over time, problems with the comparison of different species are reduced, though there may still be problems with collecting sufficient biomass. Field collections also complement the laboratory studies as a quality assurance check and by providing data on commercially important species difficult to maintain in the laboratory (e.g., lobster). In some cases, both laboratory and field assessments of tissue residues are justified by the size of a discharge or dredging operation or by the high concentration of pollutants. Guidelines on sampling designs for field surveys can be found in Green (1979), Elliott (1983), and NOAA (1988), while Holme and McIntyre (1984) contains information on the sampling techniques.

B. BIOACCUMULATION FACTORS AND ACCUMULATION FACTORS

Several approaches have been developed to predict benthic tissue residues directly from sediment concentrations thereby obviating the need for field collections or bioassays. The

simplest of these approaches is the Bioaccumulation Factor (BAF) which is:

$$BAF = Ct/Cs \qquad (1)$$

Where:

Ct = tissue concentration (ug/g dry wt)

Cs = sediment concentration (ug/g dry wt)

BAFs are empirically derived either from laboratory bioassays or field collected organisms. Assuming that BAFs were constant among species and sediments, multiplying the BAF of a compound times the sediment concentration would predict the steady-state tissue residue. BAFs are analogous to the Bioconcentration Factors (BCF) which are used to predict tissue residues from water concentrations:

$$BCF = Ct/Cw \qquad (2)$$

Where:

Cw = concentration in water (ug/g)

Although the formulas are analogous, BCFs are often calculated using wet tissue concentrations.

Sediment characteristics, such as TOC, have a major influence on the bioavailability of sediment-associated pollutants and increase the among-site variation in BAFs. BAF variability is reduced by normalizing the sediment concentrations to the TOC content (Rubinstein et al., 1983). Normalizing tissue residues to tissue lipid concentrations reduces variability in pollutant concentrations among individuals of the same species and between species (e.g., Veith, 1975; Clayton, et al., 1977).

These normalizations are combined in a simple thermodynamic-based bioaccumulation model for pollutant uptake from sediment (Lake et al., 1987; Rubinstein et al., 1987). The fundamental assumptions of this thermodynamic model are that the tissue concentration is controlled by the pollutant's physical partitioning between sediment carbon and tissue lipids and that the organism and the environment are at thermodynamic equilibrium. The method assumes that lipids in different organisms and TOC in different sediments partition pollutants in similar manners. The key value in the model is the Accumulation Factor (AF), which when multiplied by the TOC normalized sediment pollutant concentration predicts the lipid normalized tissue residue. (Note: some previous studies such as Lake et al., (1987) and McElroy and Means (1988) reported Preference Factors which are the inverse of the Accumulation Factor).

In its simplest form, the model is:

$$Ct/L = AF*(Cs/TOC) \quad (3)$$

or

$$AF = (Ct/L)/(Cs/TOC) \quad (4)$$

Where:

L = concentration of lipid in organism (g/g dry wt). (decimal fraction)

TOC = total organic carbon in sediment (g/g dry wt.) (decimal fraction)

In theory, AFs should not vary with sediment type or among species. Based on the relationship between Koc and lipid

normalized BCFs, the maximum AF for neutral organic compounds has been calculated at about 1.7 (McFarland and Clarke, 1986). Measured AFs would be lower than this maximum if metabolism of the compound by the organism is rapid or the organism fails to reach steady-state body burdens due to limited exposure durations. Measured AFs could exceed the calculated thermodynamic maximum if there is active uptake of the pollutant in the gut or if there is an increase in the pollutant's gut fugacity, driving the pollutant from the gut into the body. The pollutant fugacity in the gut could increase as the volume of food decreases during digestion or as a result of the reduction in the lipids (Gobas et al., 1988).

Laboratory and field validation of the thermodynamic partitioning model suggests that for a large number of organic pollutants, AF values do not exceed the maximum value (Ferraro et al., 1990). However, AFs for some highly lipophilic PCB congeners can exceed the theoretical maximum of 1.7 by as much as an order-of-magnitude (Rubinstein, et al., 1987). Sediments with the lowest TOCs tend to have the highest AF values (Rubinstein, et al., 1987; McElroy and Means, 1988; Ferraro et al., 1990; Lake et al., in review), which is not explained by the present model.

AFs are also dependent upon the accuracy of the lipid measurement, and total lipids can vary several fold based on the extraction technique used. As discussed in Chapter XI, we recommend the Bligh-Dyer lipid method as interim standard for AF

determinations. If another lipid extraction technique is used, a conversion factor should be provided to allow the conversion of the lipid values to chloroform-methanol extraction values.

Although laboratory and field evaluations of the AFs have shown that they are not statistically constant in all cases (Rubinstein et al., 1987; McElroy and Means, 1988; Ferraro et al., 1990; Lake et al., in review), AFs are less variable in predicting sediment uptake than BAFs (Rubinstein et al., 1987; Ferraro et al., 1990; Lake et al., in review). Because of their minimal data requirements, AFs have great potential as a cost-effective, first-order estimate of tissue residues. The predicted tissue residues can then be used for determining whether bioaccumulation tests or field surveys are needed.

For these reasons, the data required to calculate AFs should be collected and reported in all laboratory tests and field collections. Development of an AF database would be extremely useful in determining the limits of applicability of this approach, as well as generating the values for specific chemicals. After a minimum database has been collected on a compound, the AFs could be used in deriving a Sediment Quality Criterion by taking the upper 95% percentile value.

C. TOXICOKINETIC BIOACCUMULATION MODELS:

Toxicokinetic bioaccumulation models are an alternative to thermodynamic-based partitioning approaches. Toxicokinetic models assume pollutant uptake is a function of the feeding behaviors and physiological characteristics of the organism.

Most of these toxicokinetic models (e.g., Norstrom et al., 1976) assume that the tissue residue can be predicted as the sum of the uptake from each individual phase (e.g. interstitial water, ingested sediment) minus any loss due to depuration or metabolism.

In its simplest form, uptake from all phases may be expressed as:

$$dCt/dt = (Fx*CPx*EPx) - L \qquad (5)$$

Where:

dCt/dt = change in tissue residue with time.

Fx = flux of phase x through organism.

CPx = concentration of pollutant in phase x.

EPx = fraction of pollutant extracted from phase x by the organism.

L = summation of loss of pollutant through metabolism and depuration.

x = phase (W = water, F = food, S = sediment)

As an example, the uptake from water would be the product of the amount of water ventilated across the gills (FW), the pollutant concentration in the water (CPW), and the efficiency with which the pollutant is extracted from the water (EPW). As opposed to the thermodynamic model, the toxicokinetic model assumes the uptake from each route is independent and additive, so an organism exposed to two uptake phases (e.g., interstitial water and sediment) would have a higher steady-state tissue residue than an organism exposed to one phase. Toxicokinetic

models usually assume that uptake efficiency values do not change as body burdens approach steady-state and that loss (L) can be modeled as a first-order process.

These models have been used to successfully predict PCB, methylmercury, and kepone levels in marine and freshwater fish (Norstrom et al., 1976; Jensen et al., 1982; Thomann and Connolly, 1984). This approach has only recently been applied to benthic species, and has been used to model the uptake of hexachlorobenzene by a marine clam (Boese et al., 1988; Boese et al., in press; Lee et al., in press; Winsor et al., in press). A slightly different toxicokinetic model has been used to predict the uptake of various PAHs by freshwater amphipods (Landrum, 1988, 1989; Landrum and Poore, 1988). Landrum used this model to determine the relative importance of interstitial water versus ingested particulates as an uptake route for these PAHs.

In contrast to thermodynamic approaches, toxicokinetic models can predict tissue residues under non-equilibrium conditions and can account for differences in organism feeding or ventilatory behaviors due to toxic or natural effects (e.g., growth related changes). The models can also predict the time course of uptake and depuration, which can be important in certain regulatory contexts. However, the approach requires relatively sophisticated laboratory experiments to measure the input parameters. Because of the extensive data needs and the ongoing process of developing the laboratory methods, this

approach is not presently suited for the routine prediction of bioaccumulation. The toxicokinetic models are appropriate when detailed analysis of sediment or biological effects on bioaccumulation are required and as a method to test the assumptions of various Sediment Quality Criteria approaches.

APPENDIX I-2. SAFETY AND WASTE DISPOSAL

A. PERSONNEL SAFETY.

Personnel involved in any facet of bioaccumulation testing, whether sampling in the field or performing tests in the laboratory, need to be protected from exposure to toxic chemicals. Exposure to pathogens must also be considered, especially when working with sediment collected near sewage discharges. The manner in which personnel will be protected from these toxics and pathogens must be determined prior to the start of any work, keeping in mind that exposure can occur from breathing vapors, from physical contact with the skin, or ingestion of the polluted materials and/or chemicals. How one is protected depends on the type of materials and/or toxics involved and is beyond the scope of the manual. Consult the following references to determine adequate safety approaches: Sax (1984), U.S.EPA (1987b, 1988), ACGIH (1987), and U.S. Coast Guard (1986). IRIS (Integrated Risk Information System) is available to local, state, and federal public health officials through the Public Health Network (PHN) of the Public Health Foundation at (202) 898-5600 or through Dialcom, Inc., at (202) 488-0550.

B. HAZARDOUS WASTE DISPOSAL.

Hazardous waste disposal is a serious problem that must be dealt with properly. Improper shipping or disposal of toxic materials may result in environmental damage and/or serious legal consequences. The Federal Government has published regulations

for the management of hazardous waste and has given the states the option of either adopting those regulations or developing their own. If states develop their own regulations, and about half of them have, they are required to be at least as stringent as the Federal Regulations. As a handler of hazardous materials, it is your responsibility to know the pertinent regulations applicable in the state in which you are operating and to comply with them. Refer to The Bureau of National Affairs, Inc., (1986) for the citations of the Federal requirements.

APPENDIX III-1: DETERMINING NUMBER OF REPLICATES

Adequate replication is essential for determining statistically significant differences between treatments with sufficient power. If there is a question that the eight replicates recommended (Chapter III) will not provide sufficient statistical power, then the techniques in this Appendix can be used to determine the appropriate number. Determining the appropriate number of replicates requires estimates of the variability of each treatment and the minimum detectable difference. The minimum detectable difference is the smallest difference between two means, or between a mean and a constant value, that needs to be statistically distinguishable. The variability is a measure of the within-treatment variation and is expressed as a standard deviation (SD or s) or coefficient of variation (CV) and can be obtained from previous experiments or the literature. This information is needed because treatments with high variation will require more replication to distinguish differences between treatments than less variable ones. See Table III-1 for a listing of coefficient of variations for tissue residues reported for a variety of pollutants.

The number of replicates required is related to the minimum detectable difference, and detecting a 2-fold increase in tissue concentrations requires many more replicates than detecting a 100-fold increase. There are no standards for an acceptable

minimum detectable difference, but we recommend that there should be sufficient replication to detect 2-fold to 5-fold differences in tissue concentrations between two treatments (Chapter III).

Additionally, error rates for Type I and Type II errors must be chosen. A Type I error (alpha) is the probability of rejecting the null hypothesis when there is no true difference between treatment means and is usually given a value of 0.05. A Type II error (beta) is the probability of accepting the null hypothesis when there is a true difference between treatment means. As discussed in Chapter III, we recommend a beta of 0.05. This is equivalent to a power of 0.95, where the power of a test is the probability of correctly rejecting the null hypothesis.

One equation that can be used to estimate the number of replicates (n) required to detect a minimum detectable difference between two means (adapted from Sokal and Rohlf 1981) is:

$$n > 2*(s/d)^2 * (t_{alpha,v} + t_{2beta,v})^2 \qquad (1)$$

For the comparison of one mean and a constant (e.g., FDA Action Limit) the formula becomes:

$$n > (s/d)^2 * (t_{alpha,v} + t_{2beta,v})^2 \qquad (2)$$

where:

n = sample size for each treatment

s = standard deviation (often a pooled value of the two sample variances)

d = the minimum detectable difference

v = the number of degrees of freedom [v = 2*(n-1) for the comparison of two means. v = (n-1) for the comparison of a mean and a constant]

alpha = Experiment-wise or comparison-wise Type I error (see Chapter XII). If a two-tailed test is performed, each tail will consist of alpha/2. If a one-tailed test is performed, the single tail is alpha.

beta = Type II error (or 1 - power of test).

$t_{alpha,v}$ = critical value for alpha of Student's t-distribution with v degrees of freedom. (Use a two-tailed t-table for a two-tailed test and a one-tailed table for a one-tailed test.)

$t_{2beta,v}$ = critical value for 2*beta of Student's t-distribution with v degrees of freedom. (Use a two-tailed table. If a one-tailed table is used, the critical value is beta. The critical value is the same whether the test is one- or two-tailed.)

An iterative approach is used to calculate n since $t_{alpha,v}$ and $t_{2beta,v}$ are dependent on n through v. The values for $t_{alpha,v}$, $t_{2beta,v}$, alpha, beta, and v are either set by the investigator or found in tables. Therefore, only the standard deviation and the minimum detectable difference must be estimated. Although a minimum detectable difference (d) of 2 is recommended (see Chapter II), an estimate of the standard deviation will not be available in many cases. However, the ratio of the two (s/d) can be described in several ways, providing different approaches to estimating these parameters. Three methods of estimating s/d and their advantages and disadvantages are:

Method #1.

$$(s/d) = [s/(u_1-u_2)] \quad (3)$$

Where:

u_1-u_2 = difference between mean u_1 and mean u_2, or mean u_1 and a constant.

Advantages: There may be cases when an absolute difference between two numbers is of interest such as in a comparison of a measured tissue residue and a regulatory action limit.

Disadvantages: Requires an estimate of the standard deviation of the sample, a value often difficult to obtain.

Method #2.

$$(s/d) = [(CV/100)/m_1] \quad (4)$$

Where:

CV = Coefficient of Variation (expressed as a percent)

m_1 = a multiplicative factor of u_1 that is the minimum detectable difference between mean u_1 and mean u_2 (or criterion value) (e.g., if $m_1 = 5$, the minimum detectable difference between u_1 and u_2 will be five times the value of u_1).

Advantages: The CV is often easier to estimate than the standard deviation. The CV's in Table III-1 can be used as estimates if no other information is available, though it would be prudent to consider these values as the minimum estimates of variation.

Disadvantages: The value for m_1 will change whether comparisons are between control and test values or test and a criterion value. Control values (tissue residues) will tend to be low in comparison to the test values (tissue residues) while test values may be large and close to a criterion value (e.g., FDA action limits).

Method #3.

$$(s/d) = [s/(m_2*s)] = [1/m_2] \qquad (5)$$

Where:

m_2 = a multiplicative factor of s. For example, if m_2= 2, the minimum detectable difference is 2 standard deviations (i.e, u_2 will have to be 2 standard deviations from u_1 to be able to detect a difference).

Advantages: No estimates are required of the standard deviation or CV.

Disadvantages: The value of m_2 may have to vary whether comparisons are between control and test values or test and action limits.

If a comparison between more than two means is anticipated (as in the determination of steady-state conditions), see Sokal and Rohlf (1981) for a modification of this approach or Tetra Tech (1986a) for tables of estimates.

APPENDIX IV-1: ADEQUACY OF 10-DAY AND 28-DAY EXPOSURES

Ideally, organisms should be exposed to test sediments for a period sufficient to attain steady-state tissue residues. However, cost considerations often prove prohibitive to conducting tests long enough to document that steady-state has been attained. As a result, bioaccumulation tests historically have been conducted for a preset duration. Choosing a single time period is complicated by the multitude of organic pollutants and metals found in most field sediments or dredge materials, each with differing uptake kinetics. To date, a ten-day exposure to assess "bioaccumulation potential" has been the most commonly used time period for the testing of marine sediments (primarily dredge materials) (U.S. EPA/U.S. ACE, 1977). Bioaccumulation potential is the potential for any uptake of a pollutant by organisms exposed to a sediment, and the basic premise was that if there was going to be bioaccumulation it should be possible to detect it within 10 days. Thus, the original intent of the 10-day test was as qualitative rather than a quantitative measure. Since 1977, however, data from 10-day tests have frequently been extended beyond its original intent and used as a quantitative result.

Because of the wide-spread use of the 10-day exposures, it is worth assessing its utility both as qualitative measure of bioaccumulation potential and as a quantitative method to generate data for ecological and human health risk assessments. The percent of steady-state tissue residue obtained after 10 days

for several organic pollutants was used as a simple measure of accuracy (Table IV-1). To adequately assess bioaccumulation potential, the 10-day exposure should result in a sufficient percentage of the steady-state tissue residues to identify which sediments could be an environmental problem. Also, the percentage of the steady-state tissue residue obtained should be relatively consistent for the same pollutant in different species. That is, the 10-day exposure should give a strong and consistent "signal". In the quantitative risk assessments, the benthic tissue residues will be used to predict the amount of pollutants transported from the sediment to higher trophic levels, including man. A large error at the base of the food-web will result in errors throughout the analysis, especially as some of the errors may be multiplicative. As a preliminary measure, we suggest that for data to be acceptable for quantitative risk assessment, the resulting tissue residues should be within 80% of the steady-state tissue concentrations. An accuracy of 80% for each trophic step results in the prediction of tissue residues being within two-fold of the actual residues for a three step chain (i.e., sediment to benthos to demersal predator to higher predator or man; or 0.8*0.8*0.8 = 0.51)

In these studies, only 29% of the organisms approached within 80% of the steady-state level in ten days (Table IV-1). Ten-day tissue residues averaged 51% of the estimated steady-state value, and this average included some rapidly accumulated PAHs. Tissue residues of PCBs achieved after 10 days averaged

only about 25% of the steady-state values, and ranged from 100% to a low of 9%. Other environmentally important compounds with high Kow compounds, such as DDT, dioxins, and BaP, are expected to be similar to the PCBs.

Ten days is also likely to generate a relatively low percentage of the steady-state tissue residues for metals. For example, mercury levels in fish may not attain steady-state during the lifetime of the organism (River et al., 1972; Cross et al., 1973), and the minimum time for lead to attain steady-state in Mytilus edulis was greater than 230 days (Schulz-Baldes, 1974).

Based on this preliminary review, we reach several conclusions. First, a 10-day exposure generates a low percentage of the steady-state tissue residues for PCBs and presumably other high Kow organics and some heavy metals. These compounds are the most likely to represent an ecological and human health risk through bioaccumulation and biomagnification. Second, the percentage of the steady-state tissue residue obtained varies several-fold even within a single compound. Third, the amount accumulated within ten days is such a small percentage of the steady-state concentration that it may be below detection limits of standard analytical methods or may not be significantly different than control values. Thus, the 10-day exposure can result in false negatives concerning the bioaccumulation potential of a sediment. Fourth, the percentage of the steady-state tissue residues accumulated over 10 days is inadequate for

TABLE IV-1A: Information Gained and Requirements of Different Approaches to Estimating Benthic Tissue Residues

METHOD	BIOACCUM. POTENTIAL	FALSE NEG. BIOACCUM. POTENTIAL	ESTIMATES EQUILIBRIUM RESIDUE	ADDITIONAL REQUIREMENTS
Accumulation Factors	Yes	No	Yes?	Sed Conc., TOC, Lipids
10-Day Test	Yes	Yes	No	10 Days Lab Time, Tissue Conc.
28-Day Test	Yes	No	Approx. to Yes	18 Days Additional Lab Time
Kinetic Models	Yes	No	Yes	Additional Tissue Conc., Additional Lab Time?, Development of Techniques
Long-Term Exposures	Yes	No	Yes	28-70 Days Additional Lab Time, Additional Tissue Conc.

Bioaccum. Potential = Qualitative ability to detect uptake.

False Negative Bioaccum. Potential = Amount accumulated is so low that it is incorrectly concluded that no uptake will occur.

Estimates Equilibrium Residue = Tissue residue data sufficiently accurate for use in quantitative risk assessments.

Exper. Techniques = Resources devoted to determining the correct uptake and depuration periods for specific compounds and organisms
Lab Time = Laboratory time required for biological exposure
Lipids = Tissue samples analyzed for lipid content
Sed. Conc. = Sediment samples analyzed for pollutants
Tissue Conc. = Tissue samples analyzed for pollutants
TOC = Sediment samples analyzed for TOC

a quantitative risk assessment. Lastly, the 10-day exposure does not generate any additional insights into bioaccumulation potential that are not generated by use of the Accumulation Factors (see summary in Table IV-1A)

For these reasons, we conclude that an exposure duration longer than 10 days is required. Based on the use of 28 day exposures in the bioconcentration tests (ASTM, 1984), we recommend a 28-day exposure as a practical compromise between cost, data accuracy, and data utility. When 28-day organic pollutant levels were compared to observed or estimated steady-state levels (Table IV-1), steady-state tissue residues were approached (i.e.$\geq$80% of steady- state) in 76% of the tests, and the mean steady-state pollutant tissue level increased to 86% of the steady-state maximum. An average of 82% of the PCB steady-state tissue residues was obtained after 28 days, though in one instance the value was only 25% of the steady-state residue. This level of accuracy should be sufficient in nearly all cases to test for bioaccumulation potential with a reasonable level of statistical certainty. In most cases, the data should be sufficiently accurate for quantitative risk analysis. In cases when more accurate estimates are required, either a long-term exposure or a kinetic approach can be used (Chapter IV).

Besides underestimating tissue residues because of insufficient duration, single point tests can underestimate maximum tissue residues when a compound reaches a maximum value before the sampling period and then declines. For example,

phenanthrene approaches its maximum tissue residue in freshwater amphipods after about 10 days and then declines (Landrum, 1989). In this case, a 28-day test would generate a lower value than a 10-day test. Presumably, the decline is the result of an increase in the metabolic degradation rate of the pollutant, and should be most common with the lower molecular weight PAHs. Because the ability to degrade PAHs varies among taxa (Varanasi et al., 1985), a decline in tissue residues should be most pronounced with amphipods and less so with bivalves. If low molecular weight PAHs or other rapidly metabolized compounds are of interest, time series samples should be taken before day 28 (see Chapter IV).

APPENDIX IV-2: ALTERNATIVE TEST DESIGNS

A. SHORT-TERM TEST

Some compounds (e.g., volatiles) may attain steady-state in less than 28 days (see Table IV-1), so that a 28-day exposure may not be necessary. Generally, 10-day tests should be acceptable with organic compounds which have log Kow's <3 that have been spiked into sediments. Even with these compounds, a 10-day test should only be used after it has been documented to approach steady-state in phylogenetically similar species in less than ten days, or that the depuration rate (k2) in phylogenetically similar species is >0.5/day. When determining the bioaccumulation of pollutants from field sediments, however, a 28-day test should be used because nearly all field sediments contain some pollutants with slow uptake kinetics. Biotic and abiotic samples should be taken at day 0 and day 10 following the same protocol as used for the 28-day tests. If time-series biotic samples are desired, sample on days 0, 1, 3, 5, 7, 10.

B. ESTIMATING STEADY-STATE FROM UPTAKE RATES

In theory, it is possible to estimate both k1 and k2 from the uptake phase alone if the experiment continues past the point when the tissue residues begin to "bend over", indicating that depuration is sufficient to slow net uptake (Figure IV-1). This approach obviates the need to run a separate depuration experiment, as is required in kinetic approach discussed in Section C of Chapter IV. However, since both k1 and k2 are

estimated from the fitting of mathematical models, this method is less reliable than the kinetic approach which uses independent measures of k1 and k2. Nonetheless, this approach has utility when time or analytical support is limited, or if a long-term, time-series uptake test is terminated before steady-state is attained. In this design, the sampling schedule should follow closely that of the uptake phase of the kinetic approach using both uptake and depuration rates (Chapter V). Refer to Branson et al. (1975) and Foster et al. (1987) for the specifics of estimating k1 and k2.

C. GROWTH DILUTION

If test organisms grow during an experiment, growth dilution, the dilution of pollutant concentrations in the tissues by the increase in tissue mass, will occur. Taking an extreme example, if an organism doubled its weight during a depuration study, it would appear that half of pollutants had been depurated even if none of the pollutants were excreted from the organism. Without correction for growth, the depuration rate (k2) calculated from this experiment would be incorrect for an organism growing at a different rate. Many experiments have not taken growth dilution into account, which may contribute to the variation among measured depuration rates (see Niimi et al., 1981).

In 28-day experiments, growth dilution is not a problem if growth is relatively slow and kinetic rate constants are not derived from the data. However, for the kinetic approach, growth dilution can cause errors in estimating uptake and depuration parameters, resulting in errors in predicting steady-state concentrations and time to steady-state.

If substantial growth occurs during experiments to determine the rate constants, uptake rate constants will be underestimated and depuration rate constants will be overestimated. If these erroneous constants are used in the kinetic model (Equation 1 of Chapter IV) under conditions of no growth, both steady-state tissue concentrations and time to steady-state will be underestimated. Conversely, an error occurs when correct (i.e., derived under no growth) uptake and depuration rate constants are used in this kinetic model when the organisms are growing. In this case, both the steady-state concentrations and time to steady-state will be overestimated because the model does not compensate for growth dilution.

If possible, experiments should be conducted with organisms that grow very slowly or under environmental conditions that keep growth at a minimum (such as low temperatures). If growth can not be avoided, then growth dilution must be taken into consideration if a kinetic approach is used. Assuming that growth dilution is a first-order process and that growth occurs

at a constant rate, the kinetic model (Equation 1 of Chapter IV) becomes:

$$Ct(t) = k1*Cs/(k2+k3)*[1-e^{-(k2+k3)*t}] \quad (1)$$

where:

Ct = concentration in the organism at time t

Cs = concentration in the sediment

k1 = the uptake rate constant [$days^{-1}$]

k2 = the depuration rate constant [$days^{-1}$]

k3 = the growth rate constant [$days^{-1}$]

t = time [days]

The growth rate constant (k3) can be measured from the change in weight during the exposure experiment or during a separate growth experiment under similar environmental conditions. Equation 1 assumes that the k1 and k2 values are true uptake and depuration constants measured under conditions of no growth or, if growth occurs, then growth dilution was taken into account. If the depuration rate is measured while organisms are growing, the rate measured will actually be a function of growth and depuration and can be modeled as k2+k3.

Under conditions of growth, and using an estimated growth constant (k3), the maximum tissue residues becomes

$$Ct_{max} = k1*Cs/(k2+k3) \quad (2)$$

APPENDIX IV-3: CALCULATION OF TIME TO STEADY-STATE

Having an estimate of the time to reach steady-state tissue residues is very helpful in designing long-term studies and assessing the adequacy of a 28-day test. If no estimate for a pollutant in phylogenetically similar organisms is available, the time required to approach steady-state can be estimated from a linear uptake, first-order depuration model (see Chapter IV, Section C). This model is an approximation for benthic invertebrates as it was developed for fish exposed to dissolved organic contaminants.

Uptake of organic pollutants from water (dissolved phase) has been modeled in fish species using a linear uptake, first-order depuration model (Spacie and Hamelink, 1982):

$$Ct(t) = k1*Cw/k2*(1-e^{-k2*t}) \qquad (1)$$

Where:

Ct = pollutant concentration in tissue at time t

Cw = dissolved pollutant concentration in water.

k1 = uptake rate constant. [$days^{-1}$]

k2 = depuration rate constant. [$days^{-1}$]

t = time [days].

This model predicts that equilibrium would be reached only as time becomes infinite. Therefore, for practical reasons, apparent steady-state is defined here as 95% of the equilibrium

tissue residue. The time to reach steady-state can be estimated by:

$$S = \ln[1/(1.00-0.95)]/k2 = 3.0/k2 \quad (2)$$

Where:

S = time to apparent steady-state (days)

Thus, the key information is the depuration rate of the compound of interest in the test species or phylogenetically related species. Unfortunately, little of this data has been generated for benthic invertebrates. When no depuration rates are available, the depuration rate constant for organic compounds can then be estimated from the relationship between Kow and k2 for fish species (Spacie and Hamelink, 1982):

$$k2 = \text{antilog}[1.47-0.414*\log(Kow)]. \quad (3)$$

The relationship between S and k2 (using Equation 2) and between k2 and Kow (using Equation 3) is summarized in Table IV-3A. This table may be used to make a rough estimate of the exposure time to reach steady-state tissue residues if a depuration rate constant for the compound of interest from a phylogenetically similar species is available. If no depuration rate is available, then the table may be used for estimating the S of organic compounds from the Kow value. Howevér, as this data was developed from fish bioconcentration data, its applicability to the kinetics of uptake from sediment-associated pollutants is unknown and the estimated S values should be considered as minimum time periods. Also, Equation 2 does not account for

Table IV-3A: Estimated Time to Obtain 95% of Steady-State Tissue Residue

Estimated time (days) to reach 95% of pollutant steady-state tissue residue (S) and depuration rate constants (k2) calculated from octanol-water partition coefficients using a linear uptake, first-order depuration model (Spacie and Hamelink, 1982). k2 values are the amount depurated (decimal fraction of tissue residue lost per day). Note that the calculated k2 values for log Kow values <3 are expressed in hours and are based on extrapolation from the relationship between k2 and Kow, as described by Spacie and Hamelink (1982). As a result, caution should be excercised in using these particular k2 values.

Log Kow	k2	S (days)
1	0.48*	0.3
2	0.17*	0.7
3	0.07*	1.8
4	0.65	4.6
5	0.25	12
6	0.097	31
7	0.037	80
8	0.014	208
9	0.006	540

* k2 values expressed in hours, all other k2 values expressed in days.

growth dilution (Appendix IV-2). To correct for growth dilution, Equation 3 becomes

$$S = \ln[1/(1.00-0.95)]/(k2+k3) = 3.0/(k2+k3) \quad (4)$$

Where:

k3 = growth rate constant [$days^{-1}$]

Using a linear uptake, first-order depuration model to estimate exposure time to reach steady-state body burden for metals is problematical for a number of reasons. The kinetics of uptake may be dependent upon a small fraction of the total sediment metal load that is bioavailable (Luoma and Bryan, 1982). Depuration rates may be more difficult to determine, as metals bound to proteins may have very low exchange rates (Bryan, 1976). High exposure concentrations of some metals can lead to the induction of metal binding proteins, like metallothionein, which detoxify metals. These metal-protein complexes within the organism have extremely low exchange rates with the environment (Bryan, 1976). Thus the induction of metal binding proteins may result in decreased depuration rate constants in organisms exposed to the most polluted sediments. Additionally, structure-activity relationships that exist for organic pollutants (e.g., relationship between Kow and BCFs) are not well developed for metals.

APPENDIX V-1: TECHNIQUES FOR SEDIMENT MANIPULATION

In this appendix we summarize techniques to experimentally manipulate sediment for bioaccumulation tests. This is an area of much interest, and these guidelines may need revision in the future based on the ongoing research.

A. SEDIMENT SPIKING

1. Methods Used To Add Pollutants To Sediment

A variety of methods have been used to dose or "spike" sediments with pollutants. Toxicants can be added to overlying water and allowed to partition with the sediment (Breteler and Saksa, 1985; Pritchard et al., 1986), added to dry sediment and mixed by stirring or agitation (Adams et al., 1985; Foster et al., 1987), added to wet sediment and mixed by stirring or agitation (Stein et al., 1987), added with a solvent carrier, or evaporated on the sides of jars and the sediment mixed by rolling in the jars (Swartz et al., 1986; McElroy and Means 1988; Boese et al., in press). Sediments are also spiked by suspending them in aqueous solutions of the pollutants that contain carrier solvents (McLeese et al., 1980). Alternatively, carrier-free aqueous solutions of pollutants can be prepared using generator columns (Veith et al., 1975), which then can be used to spike the suspended sediment.

Solvents are often used as carriers to add hydrophobic pollutants to water and sediment. However, carriers can alter pollutant bioavailability (Dalela et al., 1979; Hughes et al.,

1983) as well as pollutant partitioning (Nkedl-Kizza et al., 1985). Although the exact effects of solvents are currently being debated, there is sufficient evidence to recommend that carrier solvents not be added directly to the sediment whenever possible. If a carrier solvent must be used, the amount should be minimized.

The effects of these various techniques on bioaccumulation have not been tested, and recent work indicates that DOC may increase proportionally with the magnitude of disturbance (mixing), continue to increase after the disturbance, and fail to return to previous DOC concentrations after 10 weeks (DeWitt, T., pers. comm., OSU, Mar. Sci. Ctr., Newport, OR). However, tests have also shown that bioaccumulation of a PCB was not affected by a long rolling time (McElroy and Means, 1988). Until standard methods are developed, it is recommended that appropriate caution be exercised in comparing spiked and field results and when comparing results from sediments spiked by different techniques.

2. Spiking Methodology

The following is a summary of the method we have used to spike two sediment types, a fine-grained sand and a silt, with hydrophobic pollutants. These techniques should work well with similar sediments, but those with drastically different properties, such as cohesive muds, may require modification. The advantages of this method are that it avoids the addition of solvents to the sediments and assures good physical mixing.

Dissolve the pollutant with an appropriate solvent and place an aliquot of the mixture into glass jars. Roll jars in a fume hood while evaporating the solvent with a gentle stream of nitrogen or purified air. After the solvent has evaporated, add wet sediment with a sufficient water content to allow adequate movement of the sediments. We have spiked 2 kg of wet sandy sediment in 1 gallon jars, and up to 13 kg of wet sandy sediment in 3 gallon jars. Leave adequate space in the jar for the sediment to roll. Cap jars and roll the sediment slurry continuously at approximately 12 rpm. The rolling apparatus can either be home-built or a commercial ball mill. Place a catch basin under the rolling apparatus to contain any contaminated sediment in case of leakage or breakage. The sediment can be rolled at room temperature or 4^{o}C, but the cooler temperature will slow microbial degradation of carbon and organic pollutants. At the end of the rolling period, place the jar in a fume hood and stir the sediment with a large Teflon^{R}-coated stainless steel or polypropylene spoon.

Appropriate solids to water ratios are essential when spiking sediment in order to assure pollutant homogeneity. Sandy sediments with water content of about 30% and fine grained sediments with water contents of 60% have been mixed successfully. However, sediments higher in organic carbon or clays may require higher water contents for successful mixing. Low water content is indicated by "balling up" of the sediment and/or inadequate movement of the sediment in the rolling jar.

The sediment needs to slide on the sides of the jar, if only slightly, while it is being rolled. If additional water is added, the excess can be removed by pipetting or siphoning after the sediment has settled. Overnight settling is usually sufficient, but longer periods may be required for particularly fine sediments. Sediment can also be centrifuged to remove the overlying water, though this may result in a sediment with too low a water content. If that occurs, water may be added back to the solids by stirring the sediment or sieving it through a 1 mm (or appropriate size) screen with a portion of the decanted water.

The mixing times required to assure a homogeneous dispersion of the spiked pollutant may vary, but sediments spiked with hydrophobic pollutants (e.g., HCB, PCBs) are physically homogeneous within one to three days. Although the pollutant concentration is homogeneous in a few days, we normally roll the sediments for ten days to allow the time for the pollutant to partition among the various sediment phases. At the end of ten days, at least 3-8 replicate samples of the spiked sediment should be analyzed to confirm pollutant homogeneity. If the sample is not homogeneous, the mixing should continue until homogeneity is achieved.

Sediment can be spiked with a water soluble (hydrophilic) compound by first dissolving the pollutant in water of appropriate salinity and then following the previously described steps of adding and rolling the sediment slurry. This procedure

differs only in that the water (solvent) is not evaporated prior to adding sediment. Hydrophilic pollutants may not require a 10 day rolling period to achieve partitioning among the water and the solid phase.

B. INCREASING SEDIMENT TOC.

The organic content of a sediment can be increased to determine effects of organic enrichment on the benthos or effects of organic matter on pollutant bioavailability. The organic content can be increased by adding sewage sludge, manure, humics, natural detritus or other organic-rich materials. Such experiments can generate important insights, but the investigator must be aware of several potential confounding factors. First, though sediments may be equal in TOC concentrations, not all organic matter reacts in the same manner. A highly labile organic source (e.g., dried baby food) is more likely to drive a sediment to anoxia than a refractory carbon (e.g., natural detritus). Second, addition of organic matter will probably change physicochemical properties such as grain size, Eh, sorption capacity, and texture of the sediment.

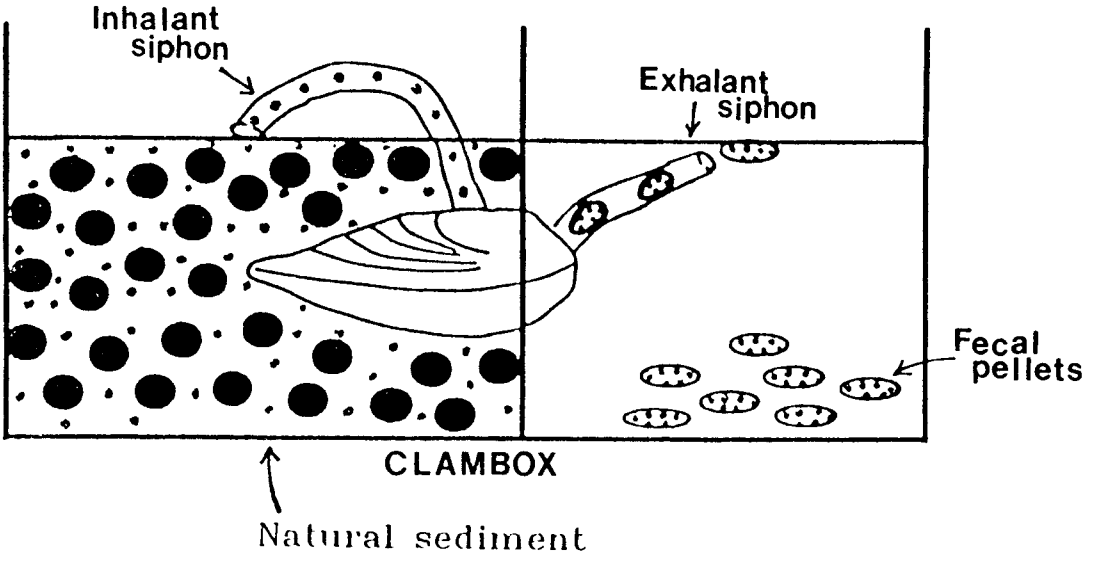
Lastly, the effect of the organic matter addition on pollutant bioavailability can vary with the feeding behavior of the test species (Lee et al., in press). Figure V-1A illustrates the effect selective feeding can have on ingested pollutant dose in natural sediment and in organically enriched sediment. In this case, the organism selects particles similar in size and

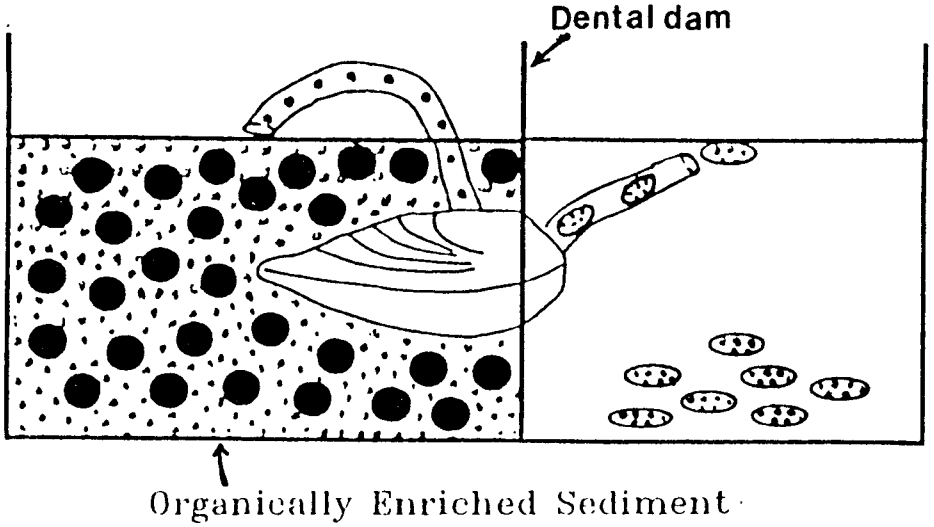
Legend to Figure V-1A: Effect of Sediment Selection on Ingested Pollutant Dose in Natural and Organically Enriched Sediments:

Figure V-1A illustrates a surface deposit-feeding clam, Macoma nasuta, in a clambox (see Appendix IX-1). The clambox separates the inhalant (left side) and exhalant (right side) siphons, allowing the collection of fecal pellets for a determination of the size and pollutant concentration of ingested particles. In the upper illustration, the clam is exposed to a natural sandy sediment, but selectively ingests the finer, high TOC particles. In the lower illustration, the clam is exposed to a sediment enriched with finer, high TOC particles. The clam ingests fine particles with a similar size and TOC content as in the sandy sediment. Because the clam ingests particles with a similar TOC content, and hence concentration of organic pollutants, the clam's ingested pollutant dose is similar in the two sediments even though the bulk sediment pollutant concentrations are dissimilar.

FIGURE V-1A

Effect of Sediment Selection on Ingested Dose in Natural and Organically Enriched Sediments





● = SAND GRAINS, LOW TOC, LOW POLLUTANT CONC.

• = ORGANIC PARTICLE, HIGH TOC, HIGH POLLUTANT CONC.

organic content in the two sediment, resulting a smaller difference in the ingested pollutant dose than would be predicted from bulk sediment analysis.

Probably the most "natural" way of augmenting sediment TOC is either by enriching a sediment with the fine fraction collected from the same sediment type (Swartz et al., 1985a) or by using the fine fraction directly (DeWitt et al., 1988; Boese et al., in press). These methods use naturally occurring organic matter, though grain size distributions of the test sediment is substantially altered. The fine fraction can be obtained by wet sieving the sediment through a series of sieves. After sieving, the fine fraction can be dewatered by allowing the solids to settle for 16-24 hours before siphoning off excess water. Excess water can also be removed by centrifugation and decanting. Similarly, other high organic materials such as sewage sludge solids can be dewatered by centrifugation and then added to the test sediment (Swartz et al., 1984).

C. DECREASING SEDIMENT POLLUTANT AND/OR TOC CONCENTRATIONS

Bulk sediment pollutant concentration and organic content can be decreased by adding control sediment, clean sand, ashed portions of the same sediment, and other inert inorganic materials, or by chemical extraction techniques. Such a manipulation can be used to reduce the toxicity of a field sediment (Swartz et al., 1989) so that its pollutant bioavailability or LC50 can be determined. These techniques, however, can unexpectedly change the pollutant sorption capacity

and the bioavailability of both metals and organics. Also, depending on the feeding behavior of the test species, addition of particles which are not ingested may have little affect on the ingested pollutant dose (see Fig. V-1A).

The least disruptive method of diluting sediments is not clear. Using a control sediment with a grain size similar to that of the test sediment will help maintain the physical characteristics of the sediment. However, by adding many partitioning sites, the addition of a control sediment may totally disrupt the distribution of the contaminant. Instead, the addition of clean sand, with few binding sites, may be a better alternative (Landrum, P., pers. comm., NOAA, Great Lakes Enviorn. Res. Lab., Ann Arbor, MI). The advantages and disadvantages of the various approaches requires further investigation.

APPENDIX VII-1: SELECTION CRITERIA FOR TEST SPECIES

A. INDIGENOUS VERSUS SURROGATE SPECIES

The first decision in choosing test species is whether to use a representative indigenous species or a surrogate species. The supposed advantage of indigenous organisms is that they are the same species which will be impacted by the dredge material or discharge. However, benthic communities can undergo drastic fluctuations in species composition in response to natural (e.g., Frankenberg and Leiper, 1977) and pollution events (Pearson and Rosenberg, 1978) and during recolonization (Rhoads et al., 1978). Because of this variation, the indigenous species chosen for laboratory testing may not be closely related phylogentically or ecologically to the species at the impacted site.

Many of the common indigenous species do not meet the criteria for use as a bioaccumulation test species, negating any advantage of using a native species. Even when an indigenous species is acceptable, established surrogate test species offer several advantages. There is considerable information on the maintenance and biology of the recommended test species. Additionally, as more tests are conducted on these species, a database will develop allowing the comparison of bioaccumulation under different environmental conditions. As ASTM (1984) has pointed out, it is more advantageous to gather detailed information on a few species rather than a smattering of information on a large number of species.

Our recommendation is to use surrogate species for routine monitoring of sediments and discharges. If there are local species which appear to meet the various criteria discussed below, they can be tested along with the recommended bioaccumulation species. If the local species prove acceptable and the results intercalibrate with the results from the standard species, the local species could be substituted for the standard species in future tests. Local species that do not meet the criteria but are of special concern (e.g., lobster) can be tested in addition to the surrogate species but should not be substituted for them.

B. REQUIRED CRITERIA:

As ingested sediment can be a major uptake route for higher Kow compounds (Landrum, 1989; Boese et al., in press), test species must ingest sediment. Using a filter-feeder, in which the only route for bedded-sediment exposure is from interstitial water, may underestimate the total uptake from sediments. Many benthic invertebrates are flexible in their feeding mode, and this requirement does not preclude the use of facultative filter-feeders (e.g., Macoma spp) as long as the only route of uptake during the exposure is from bedded sediment (i.e, no resuspended particles) and as long as the bedded sediment supplies adequate nutrition.

The requirement for a sediment ingesting species excludes obligate filter-feeders, such as Mercenaria, Mya, oysters (e.g., Crassostrea) and mussels (Mytilus spp), as well as obligate

predators such as Glycera spp. If there is concern about the human health consequences of the tissue residues in edible filter-feeding bivalves, they should be tested in addition to but not substituted for the standard test species. To accurately predict the tissue residues in filter-feeders, a resuspension exposure system is required (see Appendix IX-1).

Test species must be sufficiently pollutant resistant to survive the duration of the exposure with a minimum level of mortality. For bioconcentration tests, ASTM (1984) states that a test is unacceptable if more than 10% of the organisms "died or showed signs of disease, stress, or other adverse effects." This requirement is based on deriving BCFs for single compounds in which it is possible to control the pollutant concentration. This requirement appears too strict for testing of environmental sediments in which it is difficult to meaningfully manipulate toxicity. Nonetheless, if a pollution-sensitive species is sufficiently stressed to inhibit normal feeding, the resulting tissue residues may underestimate the amount bioaccumulated by a more hardy species. Additionally, excessive mortality can create problems in the statistical analysis of the data.

Environmentally collected sediments display a wide range of toxicities. If sediment is extremely toxic, then it may be necessary to use a highly pollution-tolerant species (e.g. Capitella spp.). As a rule, however, these pollution-tolerant species are small. Most field collected sediments will be moderately toxicity, allowing the use of the moderately

pollution-tolerant species though not the sensitive species commonly used in sediment toxicity tests (e.g., phoxocephalid amphipods). A general indication of the relative hardiness of various species can be obtained from the lists of opportunistic species listed in Pearson and Rosenberg (1978), the species used to calculate the "Infaunal Trophic Index" (Word, 1978), as well as from multispecies bioassays (Swartz et al., 1979)

C. DESIRABLE CHARACTERISTICS:

Besides the required criteria, there are a number of desirable characteristics which would make conducting the tests easier, interpreting the results more straightforward, or allow the results to be applied to a wider range of habitats.

On important characteristic, especially if repeated tests are planned, is the ease of obtaining the test species in sufficient numbers at the correct season. The ease of collecting specimens is related to a species' abundance, habitat (intertidal vs subtidal vs offshore), robustness to collection techniques, depth in the sediment, and seasonality. It is important not to underestimate the time required to collect sufficient numbers of healthy individuals. In general, it is prudent to collect twice the number required, especially polychaetes which are prone to breakage. Information on collecting and transporting specimens is given in Chapter VIII. As an alternative, test organisms may be purchased from biological supply houses or local collectors (See Appendix VIII). Local bait suppliers may sell species such

as Nereis and Callianassa.

If a large number of bioaccumulation tests will be conducted over an extended time period, culturing of test organisms may be cost effective. Culturing will provide a ready supply of organisms of known history, but maintaining a culture to supply sufficient biomass for bioaccumulation tests will be time consuming. A few sediment ingesting polychaetes (e.g., Capitella capitata and Neanthes arenaceodentata) can be cultured with relatively simple equipment (Reish and Richards, 1966; Reish, 1974, 1985; Dean and Mazurkiewicz, 1975), as can Palaemonetes (Tyler-Schroeder, 1976a,b). Although these organisms are generally suitable test species, most of the species are small, making it difficult to obtain sufficient biomass. Culture of bivalves, larger polychaetes, and most crustaceans is impractical except for experimental studies.

Regardless of how test species are obtained, they should be amenable to laboratory conditions and not require elaborate holding facilities. Fortunately, most pollutant-resistant species are relatively hardy and adaptable to laboratory conditions. Most of the bioaccumulation test species listed in Table VII-1 are reasonably easy to maintain and do not require flowing seawater.

Whether field-collected or laboratory-cultured specimens are used, gravid individuals or individuals which are likely to become gravid during a test should be avoided if possible. The reduction in tissue lipids that often occurs with spawning

(Gabbot, 1976: Davis and Wilson, 1983) can result in a corresponding reduction in the associated pollutants. Spawning may also result in unacceptable mortalities. Certain species, such as Macoma nasuta in Oregon, have a reasonably well defined spawning cycle and size at reproductive maturity, making it possible to minimize the collection of reproductive individuals. Other species, such an Neanthes virens, change appearance when reproductively mature. In extended tests, it may be impossible to completely avoid gravid individuals, though the occurrence of the reproductive state should be noted.

A very important characteristic is organism size. Test species need to be small enough to be easily maintained, yet large enough to supply sufficient biomass for chemical analysis. The amount of biomass required depends upon the analytical procedures used as well as the types of analyses required (e.g., metals, organics, lipids). At least 1 gram of wet tissue is required in nearly all cases, and commonly up to 5 grams tissue will be required. Ideally, the species should be large enough to allow chemical analysis on individuals. Chemical analyses on individuals are possible using the more "sophisticated" analytical procedures with the larger benthic organisms, such as Macoma nasuta and lug worms. Depending on the techniques, it may be impossible to conduct both metals and organic analyses on an individual, even when using large species, thereby necessitating twice as many exposure chambers if both types of pollutants are required.

An alternative approach to obtaining sufficient biomass is to composite individuals (see Chapter III). Even when compositing individuals, the size of the individual is an important consideration. It is simpler to handle and count a few larger individuals (e.g., Nereis) than dozens or even hundreds of smaller specimens (e.g., Capitella).

Species suitable for measures of sublethal stress or experimental manipulations offer the advantage that toxicokinetic or toxicological data can be collected concurrently with the bioaccumulation data. Growth is the simplest measure of sublethal stress. Measuring changes in wet weight is possible with both polychaetes and bivalves, but wet weight measurements are prone to error. Johns et al. (1989) gives techniques for conducting a growth bioassay with Neanthes arenaceodentata, some of which could be adapted to measurement of growth during bioaccumulation tests. With bivalves, growth can also be measured as changes in shell length. Although shell length has the limitation of only showing positive growth, the comparison of shell growth in treatments to the controls is a simple sublethal measure. Scope-for-growth, a measure of the amount of energy available for growth, has been used frequently as a measure of stress in mussels (Bayne et al., 1981). Scope-for-growth measurements have been conducted on infaunal polychaetes (Johns, et al., 1985) and presumably could be adapted to other bivalves and larger crustaceans, though the techniques are not simple. The arenicolid worms and the bivalves, particularly Macoma

nasuta, lend themselves to exposure chambers in which it is possible to collect ventilated water and processed sediment (i.e., feces) for bioenergetic measurements and for determinations of the pollutant uptake rates (e.g., Pelletier et al., 1988a,b; Specht and Lee, 1989). Construction of these exposure chambers is discussed in Appendix IX-1.

The more tolerant a species is to sediment, temperature, and salinity variations, the more types of sediments in which it can be used. Using a few widely adaptable species allows a direct comparison of sediment bioavailability from a variety of environments or biogeographic regions. Also, collecting and maintaining a few widely adaptable species is simpler than developing techniques for a larger number of less adaptable species. The approximate salinity and temperate ranges of potential bioaccumulation species are given in Table VII-1. These ranges are estimates of the ranges in which the organisms could be used in bioaccumulation test and are not the physiological limits. For many of the species, the ranges are based on the general literature and discussions with other researchers rather than extensive experimentation. A preliminary survival test would be advisable before initiating a large bioaccumulation test using species near the limits of the ranges given in Table VII-1.

Because the goal of bioaccumulation tests is to estimate the maximum likely tissue residues, it is important to chose species

which have a high bioaccumulation potential. Unfortunately, insufficient numbers of multi-species tests have been conducted to adequately compare the bioaccumulation potential of a range of species over a range of compounds. In general, tissue residues will be higher in species with a higher lipid contents, which can vary as much as 10-fold among species (e.g., Rubinstein et al., 1987). If PAHs are of concern, at least one test species should be a bivalve as they have a lower ability to metabolize PAHs than either polychaetes or crustaceans (Varanasi et al., 1985).

Infaunal species are preferable over epibenthic deposit-feeders because the latter are only intermittently exposed to interstitial water. Interstitial water is thought to be the major uptake route for compounds with a Kow less than about 5 (Adams, 1987) and possibly for metals as well (Assanullah et al., 1984), so the potential uptake of these compounds could be underestimated. This criterion limits the use of Palaemonetes, the only well established crustacean on the list of bioaccumulation species (Table VII-1). Infaunal crustaceans (e.g., Callianassa) have not been used extensively as bioaccumulation species, and appear to be more difficult to maintain in the laboratory than Palaemonetes.

Compatibility with other species or with the same species is important if multiple species or multiple individuals of the same species are exposed in the same chamber. Several of the nereid worms are aggressive to members of the same sex (Reish and Alosi, 1968; Johns et al., 1989). Some nereids also prey on smaller species and Palaemonetes may crop the siphons of bivalves.

D. RECOMMENDED AND SECONDARY SPECIES:

Based on these various criteria, we have identified five recommended bioaccumulation test species and another eight "secondary" test species (Table VII-1). The "recommended" species meet all or nearly all of the desired criteria and are well established bioaccumulation test species in both regulatory and experimental studies. The recommended species are the polychaetes Nereis diversicolor and Neanthes virens, and the bivalves Macoma nasuta, Macoma balthica, and Yoldia limatula. Within their tolerance levels, these species should serve as suitable test species, and we recommend using at least one of these species in all tests, at least until the suitability of other species has been demonstrated locally.

The secondary bioaccumulation species meet the required characteristics but either are deficient in one or more of the important desired characteristics and/or there is insufficient information to make a final evaluation. Some of the secondary species offer potential advantages such as including additional phylogenetic groups (i.e., crustaceans), adaptability to culturing (e.g. Neanthes arenaceodentata), and high pollution tolerance (Capitella spp). The importance of these various characteristics will depend upon the site specific situation (e.g., level of toxicity of sediment). We recognize that the list of secondary bioaccumulation species is not exhaustive, and there may be other suitable test species.

This list of recommended and secondary test species includes mostly estuarine species, reflecting both the hardiness and relative ease of collecting estuarine species. As many of these species extend into shallow marine habitats, they should serve as suitable surrogates species for near-coastal environments. There are no open ocean or brackish water (<10 ppt salinity) species included, and the species listed generally do not extend into these habitats. However, there does not seem to be a priori reason why estuarine species should underestimate the tissue residues of off-shore or brackish water species, though this assumption should be tested. Discussion of fishes or megainvertebrates is beyond the scope of this work, but a list of recommended species for field monitoring of sewage discharges can be found in Tetra Tech (1985a).

APPENDIX VIII-1: SOURCES FOR TEST ORGANISMS

BIOLOGICAL SUPPLY HOUSES

COMPANY	PERTINENT SPECIES
Gulf Specimen Company PO Box 237 Panacea, Florida 32346 904-984-5297	Nereis, Palaemonetes Uca, Callinectes
Pacific Biological Supply P.O. 536 Venice, CA 90291 213-822-5757	Palaemonetes, Capitella, Uca, Callianassa
Sea Life Supply 740 Tioga Ave. Sand City, CA 93955 408-394-0828	
Wood Hole Marine Biological Laboratory Marine Resources Dept. Woods Hole, MA 02543 508-548-3705	

OTHER SOURCES

Don Reish Dept of Biology California State U. Long Beach, CA 90840 213-985-4845	Neanthes arenaceodentata Capitella sp.
Maine Bait Co. Newcastle, Maine 04553 207-563-3000	Nereis virens
Local Bait suppliers	nereid worms, Callianassa, clams,
University Biology or Marine Sciences Depts.	wide variety

Note: Inquire as to the availability of specific species well ahead of the time the organisms are required.

APPENDIX IX-1: SPECIAL PURPOSE EXPOSURE CHAMBERS

A. CLAMBOX

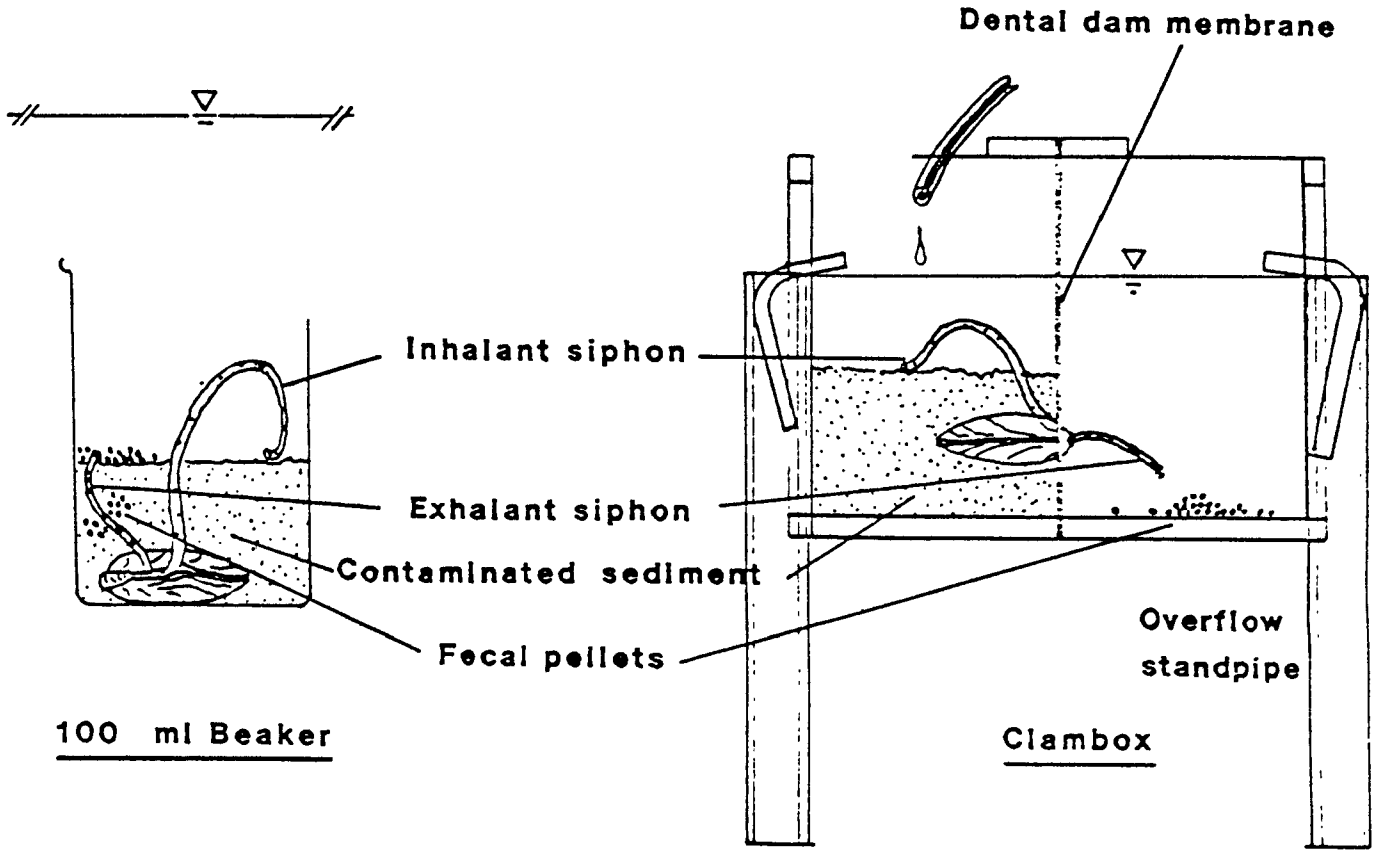
This exposure chamber is designed to separate the inhalant and exhalant siphons of sediment-ingesting clams having independent siphons (see Figure IX-1A). The technique is applicable for Macoma spp. and other tellinids, though in most bivalves the two siphons are fused together to form the "neck". The apparatus allows the isolation and collection of the feces from the parent sediment and ventilated (pumped) water from the input supply. This allows a direct measure of short- and long-term ventilation and sediment processing rates (the Fx terms of Equation 5, Appendix I-1) (Specht and Lee, 1989). By analyzing the pollutant content in the feces or the ventilated water, the amount of pollutant extracted by the clam (the EPx term of Equation 5, Appendix I-1) can be estimated. The chamber has been used to determine the efficiency of uptake of dissolved hexachlorobenzene (HCB) by the gills (Boese et al., 1988), the efficiency of HCB uptake through the gut from ingested sediment (Lee et al., in press), uptake from ventilated interstitial water (Winsor, et al., in press), and the passive sorption of HCB to the soft-tissues (Lee et al., 1988).

B. WORMTUBES

These exposure chambers are tubes open on each end, which simulate the burrow of sediment-ingesting polychaete worms such as Abarenicola pacifica and Arenicola marina. The worms pump

FIGURE IX–1A

Clambox Exposure Chamber



water and sediment in one direction through the tubes (Figure IX-1B). As with the clamboxes, the feces can be collected and separated from the parent sediment, allowing the measurement the sediment processing rate and the collection of the feces for chemical analysis. These systems have been used to study the effects of crude oil on sediment processing rates (Augenfeld, 1980) and on the uptake rate of cadmium as a function of the addition of sewage carbon to sediment (Pelletier et al., 1988b). Some versions also allow the simultaneous measurement of ventilation rate and oxygen consumption (Kristensen, E., 1981; Pelletier et al., 1988a).

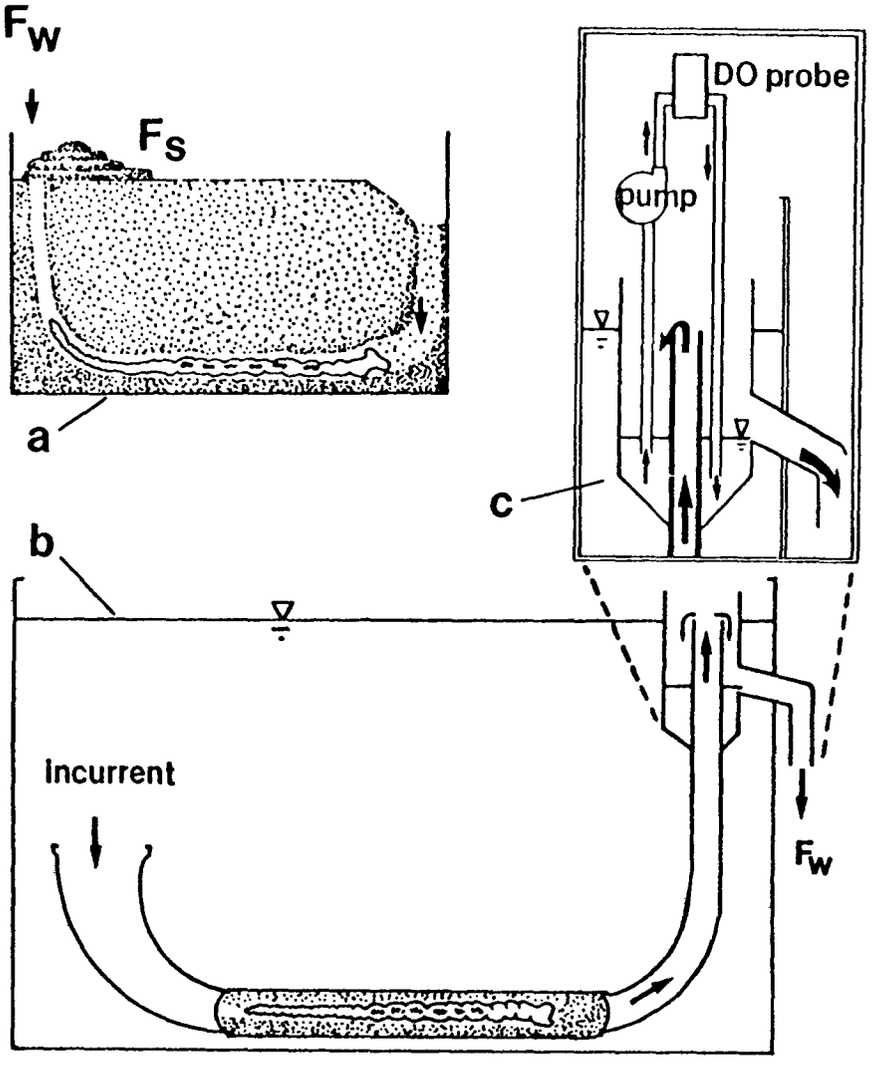
C. SEDIMENT RESUSPENSION SYSTEMS.

This flow-through device automatically maintains a constant suspended sediment load in the water column, using an electro-optical feedback mechanism (U.S. EPA, 1978) which employs an airlift dosing system, a transmissometer to measure particle concentration, and a microcomputer which calculates the dose required to achieve a programmed turbidity (Sinnett and Davis, 1983; Lake et al., 1985; Pruell et al., 1986). This system has been used in several studies on the uptake and effects of pollutants from resuspended sediments using the mussel, Mytilus edulis, and the infaunal polychaete Nephtys incisa (Lake et al., 1985; Nelson et al., 1987; Yevich et al., 1987). Other systems

for maintaining suspended sediments are given in Rubinstein et al. (1980) and Peddicord (1980). These chambers should be used when there is concern about bioaccumulation in obligate benthic filter-feeders (e.g., Mercenaria, Mya, Mytilus), or facultative filter-feeders (e.g., Macoma) via resuspended sediments. This mode of exposure is important in areas where current or wave action periodically resuspend sediments and in areas with a flocculent surface layer.

FIGURE IX-1B

Wormtube Exposure Systems



a. Worm in sediment, 1 L glass box
b. Worm in glass tube, 30 L aquarium
c. Expanded view of ventilated water collection and monitoring device

APPENDIX X-1: ADDITIONAL TECHNIQUES FOR CORRECTING FOR GUT SEDIMENT

A. MODIFICATIONS TO 24-HOUR PURGE AND DISSECTION

There are a number of other techniques or modifications to the standard 24-hour purge in control sediment (Chapter X) which should be considered in specific cases. When it is unclear whether a species is voiding all its gut contents within 24 hours, a marker "sediment" can be added to the control sediment during the purging. Marker sediments are inert particles of a contrasting color or phosphorescence under UV radiation added to the control sediment. Observation of feces composed of these marker sediments is indicator that the gut has been voided. Techniques for marking sediments for use as tracers are given in Ingle (1966). In cases when it is critical not to have any sediment in the gut, such as in certain studies of metals, it may be necessary to purge the organisms in clean water without sediment. Before using this approach, it is necessary to determine whether the test species will satisfactorily void its gut in the absence of sediment.

Another approach is to remove the gut sediment by dissection. Dissection avoids the problems with the loss of tissue pollutants during the purging, but is limited to the larger test species (e.g., Abarenicola). Care has to be taken to minimize loss of body fluids and to avoid contamination, especially with the metals. General instructions for minimizing contamination are available in Lauenstein and Young (1986).

B. CALCULATING POLLUTANT MASS OF GUT SEDIMENT

It is possible to calculate the mass of pollutant associated with the gut sediment if both the mass and the pollutant concentration of gut sediment can be estimated. For selective deposit-feeders, the pollutant concentration of the ingested sediment may be several fold greater than the concentration of the bulk sediment (see Lee et al., in press), so the bulk sediment concentration should not be used as an estimate of the gut sediment. Instead, the gut concentrations can be estimated either from the pollutant concentrations of the ingested sediment or the feces. Using the fecal pellet concentrations as the input parameter, the whole body tissue residue (Ct_w, including both the tissue and gut sediment pollutants) can be expressed as:

$$Ct_w = \frac{(M_s * CPSf) + (M_t * Ct)}{M_s + M_t} \qquad (1)$$

Expressed on a tissue residue only basis (i.e., no gut sediment), the formula becomes:

$$Ct = \frac{Ct_w * (M_s + M_t) - (CPSf * M_s)}{M_t} \qquad (2)$$

Where:

Ct_w = whole body tissue concentration (tissue and gut sediment) (ug/g)

M_s = mass of gut sediment (g)

CPSf = pollutant conc. in feces (ug/g)

M_t = mass of tissue (g)

Ct = tissue concentration without gut sediment (ug/g)

If the ingested pollutant concentration (CPSi) is used, the formula is the same except that CPSi is substituted for CPSf. Use of fecal pellet pollutant concentration underestimates the average gut pollutant content because some of the pollutants are extracted from the sediment before defecation. Conversely, ingested sediment overestimates the average gut pollutant content because some of the pollutants have been extracted. These errors are not expected to be large, but both methods could be calculated and the results averaged for the most accurate estimate. Fecal pellets can be collected for chemical analysis by using special exposure chambers such as the clambox with Macoma or wormtubes with polychaetes (see Appendix IX-1). A method to estimate ingested dose is given in Lee et al. (in press).

C. USE OF CONSERVATIVE TRACE ELEMENTS

Another approach to correcting for gut sediment is to use the concentration of a conservative, non-biologically active element as a means to determine sediment mass in the gut (Kennedy, 1986). Knowing the sediment pollutant concentration, it is then theoretically possible to calculate the amount of pollutant associated with gut sediment. Some of the conservative elements common in minerals but not typically found in more than trace amounts in tissues include silicon, aluminum, and iron (Kennedy, 1986). The difficulty with this approach is that the elemental content of gut sediment in selective deposit-feeders

may differ from that of the bulk sediment, especially if the organism selectively ingests organic rather than mineral particles. Additionally, this method will underestimate the gut pollutant mass unless the pollutant concentration of the ingested sediment (CPSi) is used rather than the bulk sediment concentration.

GLOSSARY

Accumulation Factor (AF) - Ratio of lipid normalized tissue residue to carbon normalized sediment pollutant concentration. (Appendix I-1).

ACE - Army Corps of Engineers.

Alpha - see Type I error.

Apparent Steady-State - See Steady-State.

ASTM - American Society for Testing and Materials.

BAF - See Bioaccumulation Factor.

BaP - Benzo(a)pyrene

BCF - See Bioconcentration Factor.

Bedded Sediment - Consolidated sediment (i.e., not suspended).

Beta - see Type II Error.

Bioaccumulation - Uptake from all phases, including water, food and sediment.

Bioaccumulation Factor - Ratio of tissue residue to sediment pollutant concentration. (Appendix I-1)

Bioaccumulation Potential - Qualitative assessment of whether a pollutant in a particular sediment is bioavailable. (Appendix IV-1)

Bioconcentration - Uptake from water.

Bioconcentration Factor (BCF) - Ratio of tissue residue to to water pollutant concentration. (Appendix I-1)

Block - Group of homogeneous experimental units. (Chapter III)

Coefficient of Variation - A standardized variance term; the standard deviation divided by the mean and expressed as a percent. (Chapter III)

Comparison-wise Error - Type I error applied to a single comparison of two means. Contrast with Experiment-wise error. (Chapter XII)

Compositing - The combining of separate tissue or sediment samples into a single sample. (Chapter III)

Control Sediment - Sediment with very low pollutant concentrations which is compared with reference and/or test sediments. (Chapter II)

Control Treatment - Treatment (i.e., sediment type) that is chosen to give a baseline value for comparison with results from test treatments. May consist of either control or reference sediments. (Chapter III)

Degradation - As used in the manual, it refers to the metabolic breakdown of parent pollutant by a test species. Along with depuration, it is one of the processes by which pollutants are removed from an organism.

Depuration - Loss of the parent pollutant from an organism. See Degradation.

DDT - Common environmental pollutant. Metabolites include DDD and DDE.

DOC - Dissolved organic carbon. (Chapter VI)

DOM - Dissolved organic matter. (Chapter VI)

Eh - Redox potential, which is a measure the oxidation state of a sediment. (Chapter VI)

EPA - Environmental Protection Agency

Experiment-wise Error - Type I error (alpha) chosen such that the probability of making any Type I error in a series of tests is alpha. Contrast with Comparison-wise error. (Chapter XII)

Experimental error - Variation among experimental units given the same treatment. (Chapter III and XII)

Experimental unit - Organism or organisms to which one trial of a single treatment is applied. (Chapter III)

FDA - Food and Drug Administration.

Fines - Silt-clay fraction of a sediment. (Chapter VI)

Gut Purging - Voiding of sediment contained in the gut. (Chapter X and Appendix X-1)

Ha - The alternate hypothesis. (Chapter III)

Ho - The null hypothesis. (Chapter III)

Hydrophobic pollutants - Low water solubility pollutants with a high Kow, and usually a strong tendency to bioaccumulate.

Interstitial water - Water between the particles (i.e., interstices) in sediment. (Chapter VI)

k1 - Uptake rate constant. (Chapter IV)

k2 - Depuration rate constant. (Chapter IV)

Kinetic Bioaccumulation Model - Any model that uses uptake and/or depuration rates to predict tissue residues. In this manual, it refers to the linear uptake, first-order depuration model. (Chapter IV)

Koc - Organic-carbon partitioning coefficient.

Kow - Octanol-water partitioning coefficient.

Long-Term Uptake Tests - Bioaccumulation tests with an exposure period greater than 28 days. (Chapter IV)

LOI - Loss on ignition. (Chapter VI)

Multiple Comparisons - Statistical comparison of several treatments simultaneously such as with ANOVA. (Chapter XIII)

Metabolism - see Degradation.

Minimum Detectable Difference - The smallest (absolute) difference between two means that is statistically distinguishable. (Chapter III)

NOAA - National Oceanic and Atmospheric Administration.

No Further Degradation - Approach by which a tissue residue is deemed acceptable if it is not greater than those at a reference site. (Chapter XIII)

PAH - Polyaromatic hydrocarbons.

Pairwise Comparisons - Statistical comparison of two treatments. Contrast with multiple comparisons (Chapter XII)

PCB - Polychlorinated biphenyls. Consists of over 200 congeners.

Power - Probability of detecting a difference between the treatment and control means when a true difference exists. (Chapter III)

Pseudoreplication - Incorrect assignment of replicates, often due to biased assignment of replicates. (Chapter III)

Reference sediment - Sediment used as an indicator of background pollutant levels and resulting tissue residues. May have moderate levels of pollutants. (Chapter II)

Replication - Assignment of a treatment to more than one experimental unit. (Chapter III)

Sampling unit - The fraction of the experimental unit that is to be used to measure the treatment effect. (Chapter III)

Spiking - Experimental addition of pollutants to a sediment. (Chapter V and Appendix V-1)

Standard Reference Sediment - Standardized sediment and pollutant used to determine the variability due to variation in the test organisms. (Chapter II)

Steady State - A "constant" tissue residue as determined by no statistical difference in three sampling periods (Chapter IV)

TC - Total carbon, including organic and inorganic carbon (Chapter VI)

Test Sediment - The sediment or dredge material of concern. This is the sediment on which the regulatory decision will be made. Contrast with Test Treatment. (Chapter II)

Test Treatment - Treatment that is compared to the control treatment. It may consist of a test sediment (compared to a reference or control sediment) or a reference sediment (compared to the control sediment). (Chapter III)

Thermodynamic Partitioning Bioaccumulation Model - Bioaccumulation model based on pollutant equilibrium partitioning among lipids and sediment carbon. (Appendix I-1)

Tissue residues - Pollutant concentration in the tissues.

TOC - Total organic carbon. (Chapter VI)

Toxicokinetic Bioaccumulation Model - Bioaccumulation model based on the feeding and ventilatory fluxes of the organism. (Appendix I-1)

Treatment - The procedure (type of sediment) whose effect is to be measured. (Chapter III)

TVS - Total volatile solids. (Chapter VI)

Type I Error - Rate at which Ho is rejected falsely. (Chapter III)

Type II Error - Rate at which Ho is accepted falsely. (Chapter III)

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