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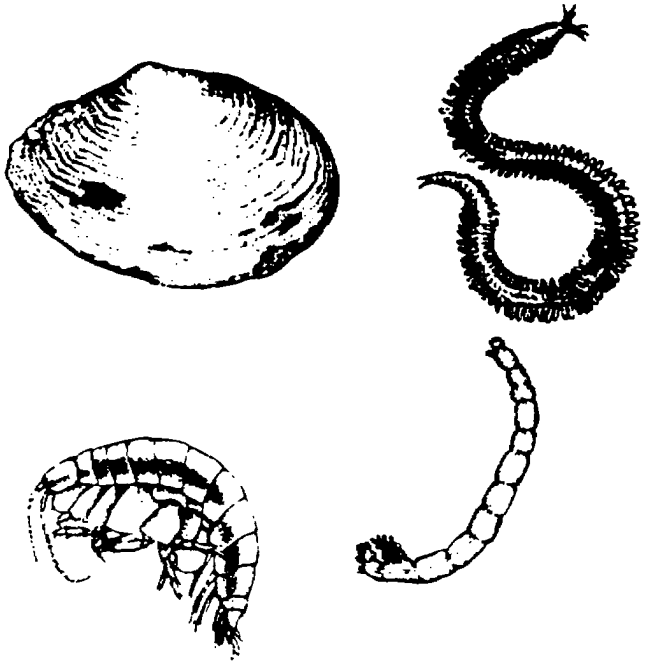
Tiered Testing Issues For Freshwater And Marine Sediments

Proceedings Of A Workshop Held In Washington, DC September 16 - 18, 1992



Proceedings

Tiered Testing Issues for Freshwater and Marine Sediments

Washington, D.C.
September 16 - 18, 1992

U.S. Environmental Protection Agency
Office of Water, Office of Science and Technology,
and Office of Research and Development
Washington, D.C.

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Introduction

This workshop was sponsored by the Environmental Protection Agency's Office of Water (OW) and the Office of Research and Development (ORD). The workshop was held to provide an opportunity for experts in sediment toxicology and staff from EPA's Regional and Headquarters program offices to discuss the development of standard freshwater and marine sediment bioassay procedures. As part of EPA's Contaminated Sediment Strategy, the Agency's program offices have agreed to develop and use consistent tests for the assessment of sediment contamination. EPA is undertaking research to address uncertainties associated with the use of bioassay test species discussed at this workshop. The results of discussions held at the workshop have been used to focus ongoing research, and to complete the development of technical guidance for conducting acute and chronic sediment bioassays and bioaccumulation tests. When completed, the technical guidance on sediment bioassay procedures will be available for use by all of the Agency's program offices. On the basis of discussions held at this workshop, the following test organisms have been selected for sediment toxicity test method development in FY93:

1. Freshwater acute toxicity tests - Hyalella azteca and Chironomus tentans
2. Freshwater bioaccumulation tests - Lumbriculus variegatus
3. Marine acute toxicity tests - Ampelisca abdita, Rhepoxynius abronius, Eohaustorius estuarius, and Leptocheirus plumulosus
4. Marine bioaccumulation tests - Macoma nasuta and Neries

If funds are available in future years, additional work will be completed on chronic test development, toxicity identification evaluation, and test development for other species.

Section one of this document contains summaries of the workshop presentations, discussions, and conclusions drawn. Section two contains the plans of work to be completed to develop sediment bioassay protocols for freshwater and marine species. These workplans were developed on the basis of discussions held at this workshop. Section three contains outlines of workshop presentations and copies of slides and graphics used by the speakers. Appendix A contains the workshop agenda, Appendix B contains a paper summarizing sediment toxicity tests being developed by Environment Canada, Appendix C contains the freshwater surveys, Appendix D contains bibliographies for a number of freshwater test species (H. azteca, C. tentans, and L. variegatus), and Appendix E contains the names and addresses of all workshop participants.

EPA's Office of Water and Office of Research and Development gratefully acknowledge the work of all workshop organizers, presenters, discussion facilitators, and participants who helped make the meeting a success. Special thanks are extended to Gary Ankley, Teresa Norberg-King, Bill Peltier, and Norm Rubinstein for compiling data on current sediment testing practices, and Tom Armitage and Beverly Baker for their work in organizing the workshop. Thanks are also extended to Elizabeth Southerland for moderating the workshop.

WORKSHOP SUMMARY

Workshop Summary

The two and one half day workshop was designed to address general issues affecting both marine and freshwater sediment testing on day one. Break-out sessions on day two focused on specific details and requirements for freshwater and marine tests. Day three consisted of reports back to the full workshop by break-out session leaders on issues discussed, conclusions, research needs, and next steps followed by an overall summary and wrap-up.

Day One

During day one of the workshop, the following talks were presented:

Introduction and Description of EPA's Sediment Strategy
Elizabeth Southerland, U.S. EPA Office of Science and Technology

Dr. Southerland welcomed participants to the workshop and stated that EPA was sponsoring this meeting to provide a forum for discussion of issues related to the standardization of sediment bioassay test methods for cross-program use. She described EPA's Draft Contaminated Sediment Management Strategy and noted that the development of consistent assessment methods was one of the major goals of the Strategy. An outline of the draft strategy was sent to more than 1000 representatives of industry, state, federal governments, and various constituent group in March of 1992. Based on comments received from the mailing and from three national forums held during the spring and summer of 1992, a final strategy will be developed and published in the Federal Register in 1993.

EPA Program Office Sediment Evaluation Needs
Thomas Armitage, U.S. EPA Office of Science and Technology

Dr. Armitage described the statutory authority and regulatory responsibility of EPA Program Offices that could use the results of sediment toxicity tests. Based on differences in regulatory responsibilities, different programs may interpret test results differently. Interpretation of individual test results may vary among programs, but standard bioassay protocols could be used by all programs. Once the method protocols have been developed, they may be used immediately by Office of Water programs, the Superfund Contract Lab Program, and EPA's Environmental Services Divisions. The Office of Pollution Prevention and Toxic Substances may begin a test rule process leading to publication of test methods in the Federal Register, and the Office of Pesticide Programs may begin their Science Advisory Panel review process. The methods to be developed will also be submitted to ASTM to begin the balloting process leading to completion of an ASTM standard method.

EPA Regional Sediment Evaluation Needs

William Peltier, U.S. EPA Region IV, Environmental Services Division - Athens, GA

Mr. Peltier discussed the EPA Regions' sediment testing needs and reminded the audience that the Regions are important clients for the methods under development. He discussed the test methods that are currently available, and noted that there are significant differences in test conditions among them. He emphasized that methods must be validated and must be run by Regional labs, state labs and consulting labs. He also noted that test conditions such as feeding, age of organisms, sediment depth, and water renewal must be clearly specified in the protocols. If pore water is to be tested, the pore extraction method must also be specified. He also raised the issue of reference and control organism survival acceptability, and discussed the possibility of using synthetic sediments for controls. Concerning species selection, he noted that there should be criteria for using regional species instead of the species selected for standardization, and that reference toxicant testing should be conducted. The question of whether reference toxicant testing should be conducted each time a toxicity test is done, or whether less frequent (weekly/monthly) testing would suffice was discussed. The importance of a good QA/QC program was emphasized. This should include laboratory evaluation, accreditation, and other checks on lab quality. Mr. Peltier also briefly discussed Regional needs for bioaccumulation testing, especially data interpretation, and closed his presentation with the reminder that Regional and state outreach was the key to success of this program.

Tiered Sediment Testing Conceptual Overview

Elizabeth Southerland, U.S. EPA Office of Science and Technology

Dr. Southerland presented an overview of tiered sediment testing. The Office of Water (OW), the Office of Pesticide Programs (OPP), the Office of Pollution Prevention and Toxic Substances (OPPTS), the Office of Solid Waste (OSW) and the Office of Emergency and Remedial Response (OERR) are all committed to the principle of consistent tiered testing outlined in the Agencywide Contaminated Sediment Strategy. Agencywide consistent testing is desirable because all EPA programs would be able to agree on whether a sediment poses an ecological or human health risk, and comparable data would be generated. It would also provide the basis for uniform cross-program decision-making within EPA. Each program, however, should retain the flexibility of deciding whether identified risks would trigger regulatory actions. Tiered testing should include a hierarchy of tests with the tests in each successive tier becoming progressively more rigorous, complex, and costly. Interpretative guidance must be developed to explain how information generated within each tier would trigger regulatory action. The interpretative guidance could be program specific describing decisions based on a weight of evidence approach, a pass/fail approach, or comparison to a reference depending on statutory and regulatory requirements. There are currently two models of sediment tiered testing used by EPA: 1) the Office of Water/US Army Corps of Engineers dredged material testing framework; and, 2) the OPP ecological risk assessment tiered testing framework. Tier one of the dredged material testing framework consists of a review of existing chemical and biological data and/or an inventory of nearby sources. In tier two, chemical data are generated and compared to water and sediment quality criteria. Tier three evaluation consists of acute toxicity and bioaccumulation testing, and a comparison of the results to a reference area. A tier four evaluation consists of

site-specific field studies. The OPP testing framework consists of acute toxicity testing in tier one, followed by chronic (early life stage) toxicity testing in tier two and further chronic toxicity testing (full life cycle) in tier three. Tier four consists of field or mesocosm testing. A tiered testing framework has not yet been chosen for Agencywide use, but some of the components that have been identified will be standardized as a result of this workshop. These components are acute and chronic toxicity bioassays, bioaccumulation tests, chemical criteria, and any others that may have ecological significance including benthic community structure evaluation, colonization rate, and *in situ* sediment testing within a mesocosm.

Summary of ASTM Activities

Chris Ingersoll, U.S. Fish and Wildlife Service, NFCR - Columbia, MO

Dr. Ingersoll of the U.S. Fish and Wildlife Service presented a summary of ASTM activities to standardize freshwater and marine sediment test methods. ASTM has not yet developed any standard methods for sediment testing but has developed guides. The most recent of these guides include: "Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediment for Toxicological Testing." (ASTM 1991 Method E1391-90); "Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs." (ASTM 1991 Method E724-89); "Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians." (ASTM 1991 E 729-88); "Standard Guide form Conducting Sediment Toxicity Tests with Marine, Estuarine and Freshwater Invertebrates." (ASTM 1991 E-47); "Standard Guide for Conducting 10-day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods." (ASTM 1991 Method E1367-90); and "Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates." (ASTM 1991 Method E1383-90). Dr. Ingersoll discussed the differences between ASTM guides and methods, and briefly described the guides listed above.

Approaches for Test Standardization; Historical Perspective and Present Guidance

Jim Lazorchak, U.S. EPA Environmental Monitoring and Systems Laboratory - Cincinnati, OH
William Telliard, U.S. EPA Office of Science and Technology

Dr. Lazorchak described a 1987 ORD document, "Guidance on Methods Standardization" which was never finalized but may serve as a framework for the methods to be standardized as a result of this workshop. There is no specific standardization process for biological methods in the document, but the process developed for chemical testing may be applied to biological testing methods development. As outlined in the document, method requirements and data quality objectives must first be established. Method selection and development is then followed by a single-laboratory evaluation involving a precision check and tests for sensitivity of method variables. This is followed by confirmatory testing by a minimum of three labs. An interim method description may then be prepared, although this has not yet been done for a biological test. A formal collaborative or round robin testing procedure may then be conducted with a minimum of six labs.

Dr. Telliard briefly described the activities of the EPA's Environmental Monitoring Management Council (EMMC) whose charter is to: 1) coordinate the Agency's environmental methods research and development activities; 2) foster consistency and simplicity in measurement methodology across regulatory programs; 3) facilitate cooperative efforts with other federal agencies, academia, industry, and other interested external parties on methods development; 4) promote and facilitate the adoption of new monitoring technology and instrumentation; and 5) evaluate the feasibility of a national laboratory accreditation program. He described the activities of the Methods Consolidation Workgroup. Their focus to date has been on methods for water, solid waste, and air, although QA/QC and biological methods will be considered by the group as well. A method validation process has not yet been developed, but a format for EMMC approved methods has been finalized.

Desirable and Necessary Attributes for Freshwater Sediment Toxicity Tests

G. Allen Burton, Wright State University - Dayton, OH
John Giesy, Michigan State University - East Lansing, MI
Chris Ingersoll, U.S. Fish and Wildlife Service, NFCR - Columbia, MO

Dr. Burton noted that desirable attributes for freshwater species and tests include: species sensitivity; reproducibility of the test; discriminatory function of the test; and "do-ability". Dr. Giesy remarked that compromises are often necessary when deciding which species to standardize. Generally, a battery of tests with several species is required to explain most of the variation in a test. Several disadvantages to the use of Chironomus tentans in sediment tests were described: these insects are relatively insensitive for use in acute toxicity tests (they are especially tolerant to metals); they do not feed directly on sediments but eat resuspended particles; genetic drift has been observed in lab-to-lab variation; there is unexplained sporadic loss of vigor in culture; and there is the potential for loss (pupation or emergence) of adults. Several other issues were identified: when to start the test; test duration; endpoints; volume of test sediments and water; food/culture medium. Similar questions about test conditions for Hyalella azteca were posed. Many of the issues highlighted by these speakers will be described in the freshwater break-out session notes.

Desirable and Necessary Attributes for Marine and Estuarine Sediment Toxicity Tests

Rick Swartz, U.S. EPA Environmental Research Laboratory - Pacific Division

Several of the same attributes and issues identified for freshwater bioassays were described for marine/estuarine tests. A major difference between marine and freshwater testing is that many marine test species are field collected not cultured. Adult organisms are used for tests rather than the young cultured organisms used in freshwater testing. This difference affects feeding regime as well as the necessity for performing routine reference toxicant testing.

Desirable and Necessary Attributes for Freshwater, Marine and Estuarine Bioaccumulation Tests

Peter Landrum, NOAA Great Lakes Environmental Research Laboratory - Ann Arbor, MI
Henry Lee II, U.S. EPA Environmental Research Laboratory - Pacific Division

The two required attributes of bioaccumulation test species are that they ingest sediment and are sufficiently pollutant resistant to survive the duration of the test with a minimum level of mortality. Other desirable attributes include: ease of collection; year-round availability; suitability to culture; adaptability to laboratory conditions; suitable size; tolerance to a wide range of sediment types; suitability for sublethal tests; ecological or economic importance; high bioaccumulation potential; and a low capability of metabolizing PAHs and other contaminants. Using an organism large enough to supply sufficient biomass for chemical analysis on individuals is especially important. Dr. Lee described the use of trophic transfer models, an equilibrium partitioning bioaccumulation model and bioenergetic models. A 28-day test length will be used for EPA test method standardization, although it was pointed out that some chemicals may not have reached steady state in that length of time. Dr. Landrum recommended using kinetic models to predict bioavailability of contaminants that are not at steady state. Two other issues raised were minimum detection levels (and guidelines) for determining tissue residue levels, and the need for interpretative guidance on test results.

Attributes of Lumbriculus variegatus that make it a good species for bioaccumulation testing are: its relatively large size (approx. 5-10 mg/organism); it is easily handled; it is tolerant to a wide range of physico-chemical conditions; it is tolerant to many contaminants; it can be used in long-term tests; standard culture and test methods have been developed; and some field validation has been done.

Identification of Long Term Research Needs

Gary Ankley, U.S. EPA Environmental Research Laboratory - Duluth, MN
Norm Rubinstein, U.S. EPA Environmental Research Laboratory - Narragansett, RI

Several long term research needs for sediment toxicity testing were identified. These include the need to develop chronic sediment toxicity tests, and the need to develop sediment toxicity identification evaluation procedures. A brief discussion of EPA's Office of Research and Development Contaminated Sediment Research Strategy is include in the end of the marine break-out session summary.

Day Two

Marine and Estuarine Sediment Testing Break-out Session

During this break-out session of the workshop, session leaders facilitated discussion of issues to be resolved for marine and estuarine sediment testing.

Objectives of the Session

Norm Rubinstein, U.S. EPA Environmental Research Laboratory - Narragansett, RI

Dr. Rubinstein identified three overall objectives for the workshop session dealing with sediment tests for marine and estuarine organisms: 1) the workshop participants should agree upon the definition of a standard method; 2) the workshop participants should reach agreement on the test species and protocols that can be standardized within the next year; and 3) workshop participants should reach agreement on the process for standardizing protocols on sediment spiking, handling, and storage.

Discussion of Marine and Estuarine Test Methods

Rick Swartz, U.S. EPA Environmental Research Laboratory - Newport, OR

Standardization Process

Dr. Swartz noted that, since exact guidance for standardizing test methods has not been developed, the ASTM process for standardizing methods should be used to provide peer review and more general participation in the process.

Identification of Uncertainties to be Addressed in Standardization Process

It was suggested that methods for sediment handling and manipulation should be addressed separately from the toxicological methods themselves. It was noted that Environment Canada is evaluating sediment handling and manipulation as a separate issue, and will soon have guidance available. There was general agreement that guidance is needed to determine how sediment should be sampled and handled in the field, transported or shipped to the laboratory, and manipulated or otherwise treated in the laboratory. It was suggested that handling and spiking could be addressed in guidance documents to accompany test method documents. There was also discussion about developing an additional standard method for experimental design that could provide specific information on such issues as sampling replicates and selection of field reference sites.

Dr. Swartz identified three categories of uncertainty that must be addressed in developing guidance: 1) limitations of the method must be described (this is distinct from information gaps); 2) issues that can be resolved on the basis of consensus should be identified (e.g. selection of the temperature for conducting tests); and 3) critical research needs that must be met should be identified (e.g. grain size tolerance). Workshop participants agreed to identify these issues for the available test organisms.

Discussion of Rhepoxynius abronius

Rick Swartz, U.S. EPA Environmental Research Laboratory - Newport, OR

Dr. Swartz indicated that Rhepoxynius has been well studied, there are 40-50 papers in the literature, and there is already one interlaboratory comparison that has been completed. Dr. Swartz noted that there are probably not many critical research needs to be filled for Rhepoxynius tests. The following consensus issues were identified for Rhepoxynius:

1. A written protocol for the standard acute toxicity test is needed. Testing procedures which must be followed should be identified.

2. Agreement must be reached on the data needed for reference toxicants. Dr. Swartz already has data on cadmium.

3. Reference sediment quality assurance/quality control requirements must be identified. Specifically, the question of the need to set a minimum desirable reference sediment survival limit was raised. If one has intermediate levels of survival in reference sediment, the difference between reference sediment and test site sediment, if detected, are difficult to interpret, if not meaningless.

4. Data are available on the seasonal sensitivity of Rhepoxynius. It should be possible to understand seasonal variability of this test species.

5. Consensus must be reached on how to ship and handle Rhepoxynius.

The need for evaluating relative sensitivity of Rhepoxynius was discussed. Reference toxicants were discussed, and it was agreed that the method guidance developed for Rhepoxynius should include information describing how to interpret sensitivity to reference toxicant tests. The utility of reference toxicants spiked in sediment was addressed. Workshop participants stated that this is a generic issue to be addressed in all of the method guidance documents to be developed.

Discussion of Ampelisca abdita

Michelle Redmond, U.S. EPA Environmental Research Laboratory - Newport, OR

Ms. Redmond described the test species and the acute toxicity test. Ampelisca abdita occurs in the high salinity range of estuaries. It is a particle feeder. Research to support the Ampelisca test is needed in a number of areas:

1. Research is needed on grain size tolerance for this species.

2. No data are available to test the sensitivity of ovigerous females. Males are not used in the test.

3. A critical research question is how to interpret control survival. Frequently problems are encountered when laboratories run the test for the first time.

Other issues were discussed. It was noted that problems have been encountered when Ampelisca is shipped to laboratories to run the test. The shipping process must be standardized. Test sensitivity has also been observed related to season. It was noted that EPA's Environmental Monitoring and Assessment Program has adopted a control survival limit of 90 percent for this species. For Hyalella a lower rate of 80 percent has been established. Salinity ranges for Ampelisca were also discussed. Some workshop participants recommended using full strength seawater for the test. The species was not recommended for tests at salinities below five parts per thousand. Workshop participants agreed that a standard method should be written for a high salinity range, and additional research can be conducted to broaden the range. There was some discussion of ammonia tolerance, and it was agreed that recent research has established the ammonia tolerance limits of Ampelisca.

Discussion of Leptocheirus plumulosus

Beth McGee, Maryland Department of the Environment - Baltimore, MD

Ms. McGee described tests using Leptocheirus. She noted that it has a wide tolerance range for both salinity (2-30 parts per thousand) and grain size (sand to silt). It was agreed that test temperature is a consensus issue, not a research issue for this species. The test is run at temperatures between 20-25 degrees C. The salinity at which tests are run should depend upon the objectives of the test. When testing the toxicity of in-place pollutants, the test site salinity may be used for the test. A photoperiod of 16:8 hours light/dark is used for the test. Juvenile and young adults (sized 3-5 mm) are tested and 20 animals are used per test chamber. Both field collected and cultured organisms are used for the test. Cadmium chloride is used as a reference toxicant for the test (96-hr LC-50).

A number of research issues were identified for Leptocheirus:

1. What are the effects of salinity on test results?

2. Must the animals be acclimated if culture or collection site salinity is different from test salinity? Tests suggest tolerance limits for sudden and extreme (i.e. 5 to 32 parts per thousand) salinity changes.

3. Are different test results obtained due to differences in culturing methods, acclimation, and salinity?

4. More data are needed to determine the sensitivity of this species to different chemicals, particularly ammonia and PAHs.

5. More interspecies comparisons are needed. Data from tests on this species could be compared to marine species, and to freshwater species capable of tolerating low salinity (i.e. Hyalella azteca).

6. More data are needed to identify sensitivity differences between laboratory and field collected animals.

7. Field validation is needed.

It was noted that there are some advantages to laboratory culture of this test species. In culture it is available at all times of the year, and cultures have been reared under known conditions. The disadvantages associated with laboratory culture include: the deleterious effects of inbreeding and the influence of culture conditions on test sensitivity. Advantages associated with field collection include: testing a natural population; the availability of large numbers of animals at low cost; the seasonal availability of size classes; accounting for the seasonal effects on sensitivity; and accounting for geographic differences in sensitivity.

A number of positive attributes of Leptocheirus as a test animal were discussed: 1) the animal is tolerant of a wide range of environmental conditions; 2) the animal has sensitivity which is comparable to other amphipods; 3) the animal is hardy and tolerant of handling, and it can be well maintained in the laboratory; and, 4) a partial life cycle test using Leptocheirus is now being developed. It was noted that investigators should be careful not to release animals in non-native regions. Workshop participants agreed that indigenous species should be used in testing when this is possible.

Note: An interlaboratory comparison has been conducted. Four laboratories participated in the acute L. plumulosus portion of a broader comparison of test methods for Chesapeake Bay methods.

Discussion of Eohaustorius estuarius

Janet Lamberson, U.S. EPA Environmental Research Laboratory - Newport, OR

The test species and acute toxicity test using Eohaustorius were described. The species occurs from central British Columbia, Canada to central California. It is a free burrowing sand dweller occurring in the top 5-10 centimeters of sediment in the upper portions of estuaries. It has an annual life cycle with recruitment occurring in the spring. The animal reproduces mostly in the spring. The young are large, and there is no problem using ovigerous females or males for testing. The recommended temperature for testing is 15 degrees C, although the animal survives well at temperatures between 5 and 25 degrees. The recommended salinity for testing is 28 parts per thousand, but the animal survives within a salinity range of 1-28 parts per thousand. Tests are conducted by introducing 20 organisms per test chamber, and the endpoints tested include: mortality, emergence, and reburial within one hour. The control sediment used is 0.5 mm sieved material taken from a collection site. The reference toxicant used with this species is cadmium chloride.

The following research issues were identified Eohaustorius:

1. Research is needed to determine the effects of light on the sensitivity of the test species.

2. The response to cadmium has varied. Additional data are needed.

3. Additional research is needed on factors affecting the sensitivity to other reference toxicants.

4. The toxicity test has not been subjected to interlaboratory comparison.

5. The toxicity test has not been subjected to rigorous field validation.

A number of advantages and disadvantages to the use of this species in toxicity testing were discussed. The following advantages were identified: it is easily collected; it can survive well in the laboratory and is sensitive over a broad range of salinities; grain size sensitivity is not in question; and it is available throughout the year and can easily be shipped. The main disadvantage to the use of the species is that it cannot be cultured.

Discussion of Lepidactylus

Ray Alden, Old Dominion University Applied Marine Science Center - Norfolk, VA

The use of this species in toxicity testing was described. The species occurs within a range from the upper Chesapeake Bay to Florida. It can be easily collected and sieved out, and is easy to handle. It has a slow rate of growth, and can reproduce slowly in the laboratory. The species can be well maintained in artificial sea water in the laboratory at a temperature of 20 degrees C under ambient light. It can be fed Artemia. The animal appears to be more sensitive

to toxicity testing in the fall, it seems to be sensitive to organic toxicants, and is tolerant of a wide range of salinities. Sediment particle tolerance may be a problem. Lower survival in fine sediment has been noted. Subadults are used for testing with 20 animals placed in each exposure chamber.

This animal appears to be a good candidate test species for acute testing but not for chronic testing, since it is slow in growing and reproducing. The amphipod is tolerant of ammonia but more research is needed to define its tolerance levels. A 16:8 light to dark photoperiod is used in most testing with this species. This has been used to simulate summer conditions. The Chesapeake Bay Program has been running tests with this species at a salinity of 15 parts per thousand, reference toxicant testing is conducted at 20 parts per thousand. The appropriate photoperiod for testing was discussed. It was noted that when the lights are turned off animals come out of their tubes and may be exposed to more fresh sediment. The availability of the species in the field was discussed. They are a dominant amphipod in North Carolina and South Carolina, and are also readily available in the Chesapeake Bay.

Discussion of Canadian Experience with Test Methods

Richard Scroggins, Environment Canada - Quebec, Canada

The Canadian experience with toxicity testing for that country's ocean dumping program was discussed. Seven amphipods, all found in Canada, were evaluated in a series of round robin tests. The testing resulted in similar responses at all laboratories for a number of species. It was mentioned that Environment Canada will recommend that organisms for testing for Atlantic and Pacific sites will be coast specific. The preferred animals for testing on the Pacific coast appear to be Rhepoxynius or Eohaustorius. For tests on the Atlantic Coast, Leptocheirus pinquis was selected.

General Discussion of Acute Amphipod Tests

A number of issues were discussed by workshop participants:

I. Experimental Design

Guidance on experimental design should be provided to support the test method guidance. A separate document could be developed that would address experimental design and interpretation of data. Workshop participants noted that it is difficult to separate effects observed in the lab from those that may or may not be observed in the field. Richard Scroggins recommended the use of five field replicates for conducting sediment tests. It was noted that appendices could be prepared for the test methods by program offices indicating how their regulatory needs could be met by the tests.

II. Commercial Availability of the Species

Workshop participants discussed the importance of commercial availability of test species. Ampelisca is available from a commercial supplier on both the east and west coasts. There are also suppliers for Eohaustorius and Rhepoxynius. Some workshop participants indicated that a directory of suppliers for test animals would be very useful.

III. Sediment Handling and Storage

It was agreed that the sediment handling and storage issue is very important. Geochemists should assist in the development of guidance in this area. Sediment handling involves a number of uncertainties. The Canadian government is trying to build on the 1990 ASTM guidelines and has formed a subcommittee of geochemists and field collection toxicologists to refine the document. The issue of storage is also important. The EPA Regional people at the session indicated that the commercial aspects of sample handling and storage are important as well.

IV. Other Issues Related to Acute Tests

Some participants noted that a separate guidance document addressing experimental design and providing guidance on sampling would be useful. It was stated that the Regions and Program Offices should provide input to address this issue. A number of comments on acute test method development were received from the Regions and Program Offices.

1. Superfund Enforcement indicated that, generally, they do not have problems using ASTM methods, and supported pursuing ASTM approval of methods developed.

2. OPPTS indicated that their real interest is in spiked sediment tests.

3. Region 1 indicated that methods developed should be subjected to interlaboratory validation.

4. Region 9 (represented by Brian Melzian currently at the Narragansett ERL) indicated that the development of methods should be accompanied by a technology transfer effort.

5. Region 4 indicated that they are looking for standard methods that can be customized to meet a particular program need. The Region will probably use a set of reference toxicants to evaluate methods.

Discussion of Chronic Test Methods

John Scott, Sciences Applications International Corporation - Narragansett, RI

A number of advantages to the use of chronic tests were identified. The tests are more sensitive than acute tests, and they may be more relevant to ecological processes. They may provide a better estimation of population level effects. A number of technical issues were identified that must be addressed to complete development of the chronic tests. These included:

1. Standardization of the age of species to be used.

2. Understanding species sensitivity in chronic tests.

3. Understanding nutritional requirements of test species.

4. Developing feeding protocols.

5. Understanding effects of sediment aging.

6. Selecting the proper endpoints and biological responses for testing.

Ted Dewitt described four species indigenous to Chesapeake Bay that were evaluated for chronic tests. He indicated that Leptocheirus appears to be the most promising one for chronic tests at this time. The species is sensitive, it reproduces easily, and it can be handled easily. The animals can be tested in "dishpans" using static renewal conditions. A water bath is used to keep the test temperature constant, and an algal (a flagellated chrysophyte) and dry food mixture is used to maintain the animals.

It was noted that in developing chronic tests, a procedure for minimizing the release of nonindigenous species should also be developed. One of the differences between a chronic test and a 10 day acute is the requirement for feeding of animals in the longer test. A number of endpoints for the chronic test were discussed. These include:

1. Mortality of adults.

2. Size of F_0 generation.

3. Fertility (number of offspring/female survivor).

4. Timing of brood emergence.

5. Reburial timing.

6. Growth.

An advantage of using Leptocheirus in chronic tests is that the animal can survive a range of sediment grain sizes and salinities.

Chris Schlekat of the Maryland Department of the Environment discussed the development of a chronic test using Leptocheirus. This species was chosen for the test because: it is endemic to the Chesapeake Bay; investigators have had success in identifying sediment toxicity with the ten day adult acute toxicity test; and it grows and reproduces well in the laboratory.

Test conditions and the results of the laboratory experiments for the Leptocheirus chronic test were described. In a comparison of juveniles and adults tested at 20 degrees C in a gradient of contaminated sediment, the juveniles were found to be more sensitive than adult Leptocheirus and juvenile Hyalella. Endpoints were evaluated for a long term chronic test using Leptocheirus. Decreased growth was measured below the lethal threshold. Tests were conducted to evaluate the influence of feeding and temperature on sensitivity to contaminated sediment. The results indicated that both factors influence the sensitivity of the animal to sediment contamination. There is uncertainty associated with the feeding of these animals at different water temperatures. However, during the tests no food buildup or increase in total organic carbon was observed. The following issues were identified to be resolved as this test is developed:

1. Appropriate test temperature.

2. Appropriate age (i.e., < 24 hours vs < 1 week old) of animal to be used for the tests.

3. Effect of source of animal on the test.

4. Effects of using cultured versus field collected animals for the tests.

5. Effects of the feeding regime used for the test.

6. Development of consistent QA/QC guidelines (reference toxicant and desired control survival).

7. Interaction between nutrition and toxicological sensitivity.

8. Effects of other variables on the test (e.g. salinity and grain size).

9. Simplification of the methodologies used for the test (e.g. microalgal culturing).

10. Comparison of the relative sensitivity of endpoints between species.

Michelle Redmond discussed the use of Ampelisca in chronic testing. This species has a growth curve similar to Leptocheirus. A number of experiments have been conducted to improve the reproduction of this animal in the laboratory. The effects of differences in population density, aeration, and other variables has been investigated. Success in maintaining the cultures has been variable. Experiments indicate that aeration is necessary to conduct the sediment test, and that survival of juvenile offspring obtained from cultured females is greater. It will be necessary to look at shipping and handling to determine if this is related to decreased survival. The following observations were made based on test results:

1. Short term and chronic sublethal tests can be conducted using Ampelisca.

2. Known age animals can be released from the females.

3. Newly released or 10 day old animals can be used for the test.

4. Problems of low reproduction and poor survival were encountered, perhaps related to shipping and handling.

5. A flow through system and changes in photoperiod may be necessary to conduct the test.

6. Nutrition may be a factor affecting test results.

Discussion on Bioaccumulation Testing

Henry Lee, U.S. EPA Environmental Research Laboratory - Newport, RI

The development of a standard 28 day bioaccumulation method for two species, Neries and Macoma, was discussed. A draft guidance manual on bedded sediment bioaccumulation tests has been produced by the EPA Newport Laboratory. Workshop participants agreed that, given the current state of knowledge, these test protocols can now be written. Some additional research must be completed. Longer term tests and kinetic models will also provide tools to evaluate bioaccumulation. It was recommended that a series of round robin tests be completed using Neries and Macoma to provide some indication of the precision to be obtained with the tests. The round robin experiments will not be cheap because of the requirement for tissue analysis. It would probably be necessary to use spiked sediment with a high Kow compound.

Region 9 has expressed interest in using their own species for the bioaccumulation test. Workshop participants noted that it will be necessary to develop guidance on interpretation of bioaccumulation results. This will depend upon whether human health or ecological health is of concern. Human health may be the most significant endpoint to be addressed.

It was agreed that the protocol will be developed as follows:

1. Protocol will be for a 28 day solid phase test.

2. Test will use a flow through seawater system.

3. Test protocol will describe test organism acclimation, maintenance, introduction, data recording, removal, gut depuration, and sample preservation.

With this protocol, 80 percent of the steady state level will be reached. There was some discussion of gut depuration. In cases where bioavailability is the issue, gut depuration may not be warranted. Analytical results may be 20 percent higher when the gut is not purged. If statistical comparisons are used for regulation, this may make a difference. If regulations are based on a factor of two or higher, gut depuration will probably not make a difference.

Discussion of Office of Research and Development Contaminated Sediment Research Strategy

Norm Rubinstein, U.S. EPA Environmental Research Laboratory - Narragansett, RI

The EPA Office of Research and Development (ORD) Contaminated Sediment Research Strategy was discussed. ORD has restructured the research planning process. Contaminated sediment is an ORD research issue, and an issue paper has been developed to outline the research program. Research will focus on developing technically valid assessment approaches, development of sediment criteria, development of remediation technologies, and monitoring programs (EMAP, ARCS, and NEP). Considerable research will be conducted on field validation of the equilibrium partitioning approach for the development of sediment quality criteria. Research will also be conducted on evaluation of ecological risk.

Day Two

Freshwater Sediment Testing Break-Out Session

During this break-out session of the workshop, session leaders facilitated discussion of issues to be resolved for freshwater sediment testing.

Objectives of the Sessions

Gary Ankley, U.S. EPA Environmental Research Lab - Duluth, MN

As a preliminary step for the second day of the workshop, a questionnaire was sent out to workshop invitees and other selected researchers on July 13, 1992. The primary information requested was about culturing and testing for three freshwater species (H. azteca, C. tentans, and L. variegatus). The survey was done in order to assemble as much information as possible, before the workshop, to be more effective in discriminating the various approaches being used by researchers conducting tests with the three species. These responses were summarized by the Environmental Research Laboratory in Duluth, and the major culturing and testing issues were identified and prioritized for discussion at this workshop. The discussion issues for culturing and testing of the three species listed were ranked in order of importance to development of standard methods and based on the similarity of the issues across all tests. For example, the culturing issues ranked for H. azteca were: known age culture systems; feeding regimes; water for culturing; flow-through versus static systems; quality assurance/quality control (e.g., reproduction levels, reference toxicants); and genetic drift/strain differences. The testing issues for Hyalella were: test length/endpoint; organism age to start the test; water renewal frequency (volume, method); interpreting the effect of sediment variable on test results (e.g., organic carbon, particle size); feeding levels/appropriateness in sediment tests; and quality assurance/quality control (criteria for acceptable tests). These were generally the same issues for C. tentans and L. variegatus.

This session was designed to: (1) identify freshwater sediment toxicity tests as candidates for standardization within the next year; and (2) to explore specific technical issues associated with each test for the purposes of reaching a general consensus or for identifying areas requiring future research. Each issue identified during the session was discussed and, where possible, a consensus was reached for development of standard protocols.

Development of a Standard Testing Protocol for Hyalella azteca

Teresa Norberg-King, U.S. EPA Environmental Research Lab - Duluth, MN

Twenty one responses to the survey were received, and eighteen laboratories (see Appendix D) reported information on Hyalella azteca. The summary of the survey responses are as follows:

I. Summary for *Hyalella azteca*. The most common procedural response is underlined and when no item is underlined it indicates no single most common response.

Culture Methods

Flow:	Static vs. renewal
Temperature:	19 to 25°C (23°C)
Light:	16:8 photoperiod; 50 to 100 ft. candles
Chamber:	1 L to 40 L
Age animals:	Known age vs. mixed age
Freq. restart:	Monthly, every 2 months
Water Quality:	Natural vs. reconstituted
Source of Strains:	ERL-Duluth, ERL-Corvalis, Burlington, Michigan State (most cultured in moderately hard water or hard water)
Aeration:	Moderate
Feeding:	Leaves, Tetramin®, rabbit chow, diatoms, yeast, wheat grass, *Chlorella*, alfalfa, Nutrafin®, YCT, paper towels, *Selenastrum*, *Ankistrodesmus*, brine shrimp, aquatic plants, sediment. Feed 2 to 3 times/week typical.
Substrate:	Leaves, nylon mesh, cotton gauze, 3-M web plastic, paper towels
Reference Toxicants:	Cd, Cu, KCl, Zn, NaCl, Cr (water-only exposures)

B. Testing Procedures

Flow:	Static vs. renewal.
Aeration:	None or moderate
Temperature:	20 to 25°C (20°C)
Light:	16:8 photoperiod; 25 to 50 ft. candles
Chamber:	30 mL to 1 L (250 to 300 mL)
Sed. ratio:	1:1 to 1:4 ratio sediment:water
Age animals:	Known age (0 to 7 d, 7 to 14 d) vs. mixed age (size about 7 to 14 d) (sieved)
No. animals:	5 to 20/beaker (10/beaker)
No. reps:	2 to 10/treatment (3 to 5/treatment)
Duration:	2- to 28-d (10-d)
Feeding:	None, Rabbit Chow, YCT, maple leaves, Tetramin®
Endpoints:	Survival, length, weight, sexual maturation (males), young production, bioaccumulation
Acceptability:	Survival (80%), length, weight

Development of a Standard Testing Protocol for Chironomus tentans

Robert Hoke, AScI - Duluth, MN

Twenty one responses to the survey were received, and twelve laboratories (see Appendix D) reported information on Chironomus tentans. The summary of the survey responses are as follows:

I. Summary for *Chironomus tentans*. The most common procedure is underlined and when no item is underlined it indicates no single most common response.

A. Culture Methods

Flow:	Static vs. renewal
Temperature:	19 to 25°C (23°C)
Light:	16:8 photoperiod; 50 to 120 ft. candles
Chamber:	1 L to 40 L
Age animals:	Known age vs. mixed age
Freq. restart:	2x week to every 6 months
Age restart org:	egg cases to ≤24 h old larvae
Water Quality:	Natural vs. reconstituted
Aeration:	Moderate
Feeding:	Tetramin®, Nutrafin®, YCT and algae, alfalfa and Tetramin Feed daily to 3x/week
Substrate:	paper towels (bleached or unbleached); sand
Reference Toxicants:	Cu, NaCl, Cd, KCl (water-only exposures)

B. Testing Procedures

Flow:	Static vs. renewal
Aeration:	None or moderate
Temperature:	20 to 25°C (23°C)
Light:	16:8 photoperiod; 25 to 120 ft. candles
Chamber:	50 mL to 2 L
Sed. ratio:	1:1 to 1:4 ratio sediment:water
Age animals:	Known age (0 to 16 d; 10 to 14 d)
No. animals:	15 to 80/beaker (10 to 15/beaker)
No. reps:	2-15 (3 to 4)
Duration:	2 to 14-d (10-d)
Feeding:	trout chow, Tetrafin®, YCT
Endpoints:	Survival, weight
Acceptability:	Survival (70%), weight (dry weight)

Development of a Standard Testing Protocol for Lumbriculus variegatus

Peter Landrum, NOAA Great Lakes Environmental Research Laboratory - Ann Arbor, MI

Twenty one responses to the survey were received, and five laboratories (see Appendix D) reported information on Lumbriculus variegatus. The summary of the survey responses are as follows:

I. Summary for *Lumbriculus variegatus*. The most common procedure is underlined and when no item is underlined it indicates no single most common response.

A. Culture Methods

Flow:	Static vs. renewal
Temperature:	22 to 24°C
Light:	16:8 photoperiod; intensity unspecified
Chamber:	1 L to 40 L
Age animals:	mixed
Freq. restart:	Monthly, every 2 months
Water Quality:	Natural vs. reconstituted
Aeration:	Moderate
Feeding:	Frozen silver cup trout chow, salmon starter, sediment, Tetramin®, yeast, wheat grass, *Chlorella*, alfalfa, Nutrafin®, YCT, paper towels food. Feed 2 to 3 times/week typical.
Substrate:	paper towels, sediment
Reference Toxicant:	no reference toxicants specified

B. Testing Procedure

Flow:	Static vs. renewal
Aeration:	None or moderate
Temperature:	10 to 23°C
Light:	16:6 photoperiod
Sed. Ratio:	1:1 to 1:4 ratio sediment: water (sediment volumes should be adequate to allow feeding and burrowing)
Age animals:	Adults, 3.8 cm.
No. animals:	Adequate number to provide tissue mass for analysis of residue of concern
No. reps:	4 to 5/treatment
Duration:	10 to 28 d
Endpoints:	Bioaccumulation
Feeding:	None
Acceptability:	Adequate tissue mass for residue analysis

Workgroup Recommendations

Following the presentations of the culturing and testing survey results, there was an open dialogue about the key issues. Generally for most of these issues, workgroup participants offered suggestions about currently used versus preferred approaches. The workgroup arrived at a consensus on several culturing and testing specifics. Where it was not possible to make a decision because of lack of information, the group identified research items that need further consideration before a specific decisions could be made. The workgroup's consensus and research issues for the various species tests are summarized below.

In developing guidance for culturing freshwater species to be included in the EPA methods manual for sediment tests, it was generally agreed that there was not just one method that may be used to culture the three species. It was generally concluded that success of the tests would rely heavily on the health of the culture from which the animals were taken for testing. That is, having healthy animals of known quality and age for testing was deemed to be the key consideration relative to culture conditions. Therefore, a *performance-based criteria* approach was selected as the preferred method through which individual laboratories should evaluate their culture protocol rather than by a *control-based criteria* approach. This method was chosen to allow each laboratory to optimize their own, perhaps unique, culture techniques, and meet certain quality control monitoring steps in cultures, while providing organisms that would produce reliable, comparable test results.

Hyalella azteca:

Performance-based culturing criteria for Hyalella azteca would include:

1. Laboratories must perform monthly water-only reference toxicant tests to assess the health of their culture organisms or the organisms they purchase from other laboratories. The reference toxicant test should be performed as a 96 h water-only test. Laboratories should also evaluate the slope of their LC50 curve for each reference toxicant test. Results of these monthly tests then would be entered into "control" charts; test results greater than two standard deviations from the mean LC50 might indicate a cause for concern that the cultures are unhealthy.

2. Laboratories should track the parental survival because it is important to know the reproduction trends of reproduction for the cultures; it was suggested that this be developed in a manner similar to that used as the control chart for reference toxicant data.

3. Laboratories should routinely measure and record the following culture water chemical parameters: pH; D.O.; hardness; alkalinity; and ammonia. Again it was suggested that this be developed in a manner to that used as the control chart for reference toxicant data.

4. Laboratories should characterize and monitor the quality of the food they use in terms of nutrient content and contamination.

5. Laboratories should keep records of their culture restart interval and keep accurate records on the age of the brood animals and, as far as possible, track the source of test animal to the age of the brood animals. Physiological parameters such as lipid content also might be considered as a culture parameter to determine the health of the organisms.

6. Laboratories should develop standard operating procedures to ensure that their cultures are renewed and monitored on a regular and standardized manner.

Given that the *performance-based culture criteria* will be part of the requirements for the test performance, the major *consensus* items for the Hyalella azteca test protocol were as follows:

1. The use of the *performance-based culture criteria* for cultures will be used to judge the health of the animals in the toxicity test, along with the acceptability criteria. The test acceptability criteria were agreed upon as 80% control survival in a 10 d test as well as acceptable water chemistry parameters over the course of the test.

2. The renewal of the overlying water in the sediment test at a rate of 1.25 to 4 volumes/day was agreed upon. The specific procedures were not specified for the water renewals; however, the intervals should be evenly spaced over 24 h.

3. The age of the animals for testing was 0 to 14 d. However, the workgroup recognized that older animals (7 to 14 d) are easier to recover in the whole sediment compared to 0-7 d old animals. This necessitates the need for known age culture systems.

4. The test length discussed ranged from 7-28 d, yet since the majority of the workgroup participants were using 10-14 d for the survival endpoint, consensus was to use the 10 d test period. This test duration may allow the growth endpoint to be measured after 10 d, but more research would be needed before this could be added as a criterion for test acceptability.

5. The number of animals needed per concentration and the number of replicates needed was discussed. The choice of each (replicates, number per concentration) will depend in part on the probability level selected, and the type of statistical analysis. When variability remains constant, the sensitivity of the test increases as the replicates increase. Further evaluation of the appropriate alpha (α) (significance level) and beta (β) (power of the test) for the Type I and Type II errors is needed along with consideration of the delta (δ) (minimum detectable difference). It was the group consensus that this analysis could be done with existing data.

6. Although various size test chambers are used, it appeared that numerous researchers were looking for smaller test volumes and test chambers and use of 250 to 300 ml beakers with 50 to 100 ml of sediment for 10 to 20 animals each was agreed upon. Larger chambers have been used in the past but because space can be a limiting factor or recovery of animals from larger volumes of sediment may be lower, it was the groups' consensus that smaller chambers would be acceptable. The minimum amount of sediment needed to use in tests may need further consideration.

7. Feeding during the 10-d survival exposure was deemed necessary. Otherwise, acceptable survival cannot be achieved with H. azteca. One food should be used for the standard method. However, the decision of what the food should be and the quantity of it are yet to be determined.

8. The test temperature was agreed to be 23 $\pm$ 2°C as most laboratories could accommodate that now.

9. The analysis of abiotic factors that may affect test results is needed. This includes issues such as the sediment grain size and/or organic carbon content which may affect organism survival and/or growth. It was the group consensus that guidance on this issue could be developed based upon existing data sets developed in H. azteca tests with a range of clean sediments.

The following **research needs** were identified for H. azteca.

1. *Additional research* is needed to develop approaches to produce known age animals for testing. Alternatively, the manuals would state that if cultures were sieved to obtain test organisms, the age of the animals retained by the sieve must have been previously determined in that culture system.

2. *Additional research* is needed to evaluate the sensitivity of the various strains of H. azteca to assess whether there is significant genetic drift depending on the waters that animals were obtained from and/or the length of time they have been in monocultures.

3. *Additional research* is needed to develop the standard food for the tests that will provide minimal organic carbon input while providing sufficient nutrition.

4. *Additional research* on the age of the test animals that can be used and still meet the minimum required recovery is needed. Testing underway at Duluth may help to decide whether 0-7 d organisms are any different in sensitivity than the 7-14 d old organisms which are easier to recover from whole sediment tests.

5. *Additional research* is needed on the abiotic factors that may affect test results (e.g., organic carbon, particle size). Data from Canada (Burlington), Duluth, the ARCS program, and Mississippi should assist in making the decision.

6. *Additional research* is needed to determine the significance of various funding regimes on the toxicity test. Feeding may alter the exposure through reduced uptake and enhanced elimination of the contaminants.

Chironomus tentans/riparius:

The workgroup *consensus* was that the C. riparius could be included in the C. tentans protocols; these species are fairly similiar.

Most of the *performance-based culture criteria* listed for H. azteca are relevant to the Chironomus spp. In addition to the chironomids, it is important that the following be considered in the *performance-based criteria*:

1. Laboratories should measure and record the dry weight of at least one larval stage (e.g., day 12) for the purpose of maintaining control charts for each culture. This is a similar parameter to young production counts for H. azteca.

2. Laboratories should record the time to first emergence for each culture and keep this data in a control chart. Fluctuations in time to emergence often precede loss of vigor in chironomid cultures.

3. Laboratories must perform monthly water-only reference toxicant tests to assess the health of their culture organisms or the organisms they purchase from other laboratories. The reference toxicant test should be performed as a 96 h water-only test. Laboratories should also evaluate the slope of their LC50 curve for each reference toxicant test. Results of these monthly tests then would be entered into "control" charts; test results greater than two standard deviations from the mean LC50 might indicate a cause for concern that the cultures are unhealthy.

4. Laboratories should routinely measure and record the following culture water chemical parameters: pH; D.O.; hardness; alkalinity; and ammonia. Again, it was suggested that this be developed in manner similar to that used as the control chart for reference toxicant data.

5. Laboratories should characterize and monitor the quality of the food they use in terms of nutrient content and contamination.

6. Laboratories should keep records of their culture restart interval and keep accurate records on the age of the brood animals and, as far as possible, track the source of test animals to the age of the brood animals.

7. Laboratories should develop standard operating procedures to ensure that their cultures are renewed and monitored on a regular and standardized manner. Physiological parameters such as lipid content also might be considered as a culture parameter to determine the health of the organisms.

Given that the *performance-based criteria* for the cultures will be part of the requirements for the test performance, the major *consensus* items for the C. tentans test protocol are as follows:

1. The age of the test animals should be: 8 to 12 d old for C. tentans and 6-8 d old for C. riparius.

2. The use of the *performance-based criteria* for cultures will be used to judge health of the animals in the toxicity test, along with the acceptability criteria. The test acceptability criteria were agreed upon as 70% control survival and a growth weight requirement (to be determined) in a 10 d test as well as acceptable water chemistry parameters over the course of the test.

3. The renewal of the overlying water in the sediment test at a rate of 1.25 to 4 volumes/day was agreed upon. The specific procedures were not specified for the water renewals; however, the intervals should be evenly spaced over 24 h.

4. The test length discussed ranged from 7-21 d, yet since the majority of the workgroup participants were using 10-14 d for the survival and growth endpoint, consensus was to use the 10 d test period. This test duration may allow the growth endpoint to be measured after 10 d, but more research would be needed before this could be added as a criteria for test acceptability.

5. The number of animals needed per concentration and the number of replicates needed was discussed. The choice of each (replicates, number per concentration) will depend in part on the probability level selected, and the type of statistical analysis. When variability remains constant, the sensitivity of the test increases as the replicates increase. Further evaluation of the appropriate alpha (α) (significance level) and beta (β) (power of the test) for the Type I and Type II errors is needed along with consideration of the delta (δ) (minimum detectable difference). It was the group consensus that this analysis could be done with existing data.

6. Although various size test chambers are used, it appeared that numerous researchers were looking for smaller test volumes and test chambers and use of 250 to 300 ml beakers with 50 to 100 ml of sediment for 10 to 20 animals each was agreed upon. Larger chambers have been used in the past but because space can be a limiting factor or larger chambers, it was the groups' consensus that smaller chambers would be acceptable.

7. Feeding during the 10 d survival exposure was deemed necessary. Otherwise, acceptable survival cannot be achieved with C. tentans. One food should be used for the standard method. However, the decision of what the food should be and the quantity of it are yet to be determined.

8. The test temperature was agreed to be 23 $\pm$ 2°C as most laboratories could accommodate that now.

9. The analysis of abiotic factors that may affect test results is needed. This includes issues such as the sediment grain size and/or organic carbon content which may affect organism survival and/or growth. It was the group consensus that guidance on this issue could be developed based upon existing data sets developed in C. tentans tests with a range of clean sediments.

The following **research needs** were identified for Chironomus spp. toxicity tests as well as those for the amphipod test above.

1. *Additional research* is needed on the abiotic factors that may affect test results (e.g., organic carbon, particle size). Data from Canada (Burlington), Duluth, the ARCS program, and Mississippi should assist in making the decision.

2. *Additional research* is needed to develop approaches to produce known age animals for testing. Alternatively, the manuals would state that if cultures were sieved to obtain test organisms, the age of the animals retained by the sieve must have been previously determined in that culture system.

3. *Additional research* is needed to develop the relative sensitivity data of midges and other taxa.

4. *Additional research* is needed to develop the standard food for the tests that will provide minimal organic carbon input while providing sufficient nutrition.

5. *Additional research* on the age of the test animals that can be used and still meet the minimum required recovery is needed.

Lumbriculus variegatus:

Many of the *performance-based criteria* described above for H. azteca are relevant to L. variegatus. Additional culture considerations follow:

1. Laboratories must perform monthly water-only reference toxicant tests to assess the health of their culture organisms or the organisms they purchase from other laboratories. The reference toxicant test should be performed as a 96 h water-only test. Laboratories should also evaluate the slope of their LC50 curve for each reference toxicant test. Results of these monthly tests then would be entered into "control" charts; test results greater than two standard deviations from the mean LC50 might indicate a cause for concern that the cultures are unhealthy.

2. Laboratories should monitor and record the frequency with which the population is doubling. Again, this might be done using the control chart concept.

3. Physiological parameters such as lipid content also might be considered as a culture parameter to determine the health of the organisms.

4. Laboratories should routinely measure and record the following culture water chemical parameters: pH; D.O.; hardness; alkalinity; and ammonia. Again it was suggested that this be developed in manner similar to that used as the control chart for reference toxicant data.

5. Laboratories should characterize and monitor the quality of food they use in terms of nutrient content and contamination, particularly for the compounds to be evaluated for bioaccumulation.

6. Laboratories should keep records of their culture restart interval.

7. Laboratories should develop standard operating procedures to ensure that their cultures are renewed and monitored on a regular and standardized manner.

Given that the *performance-based culture criteria* will be part of the requirements for the oligochaete test performance, the major *consensus* items for the test protocol are as follows along with the various parameters of importance for the test method as discussed for H. azteca above:

1. For the time being, 28 d tests are recommended; however, ongoing research may indicate that shorter tests could be used. The use of the *performance-based culture criteria* for cultures will be used to judge the health of the animals in the bioaccumulation test, along with the acceptability criteria. The test acceptability criteria will include acceptable water chemistry parameters over the course of the test.

2. During tests, the organisms will not have any food added to the test chambers.

3. The desirable chamber size is more variable than for the previous two species and is dependent upon the organism/sediment carbon ratios needed.

4. The number of organisms used per replicate will depend on obtaining an adequate tissue mass for analysis of compounds of concern. Also, it was suggested that the ratio of organism carbon/sediment organic carbon in the test systems should be on the order of greater than 1/10 to 1/100.

5. The renewal of the overlying water in the sediment test at a rate of 1.25 to 4 volumes/day was agreed upon. The specific procedures were not specified for the water renewals; however, the intervals should be evenly spaced over 24 h.

6. The test temperature was agreed to be 23 $\pm$ 2°C as most laboratories could accommodate that now.

7. The analysis of abiotic factors that may affect test results is needed. This includes issues such as the sediment grain size and/or organic carbon content.

The following **research needs** were identified for L. variegatus.

1. *Additional research* to determine the minimum and optimal time of exposure for bioaccumulative compounds is needed. This would include tests that would evaluate whether a shorter test (7-10 d) might be possible rather than the 28 d test.

2. *Additional research* to evaluate gut purging times in relation to elimination of contaminants is needed. Evaluate the appropriate time to allow for gut purging and whether this is contingent on chemical class.

3. *Additional research* to evaluate kinetics of the uptake/depuration for chemicals over a wide range of Kow's.

4. *Additional research* is needed to evaluate optimal organism carbon/sediment ratios for tests.

5. *Additional research* is needed to determine the potential of "sediment avoidance" on bioaccumulation of contaminants by L. variegatus.

6. *Additional research* is needed on organism loading and doubling of populations during kinetic studies.

Day Three

Conclusions and Next Steps

Dr. Southerland opened the session. She stated that, on the basis of discussion held at this workshop, EPA will develop standard test protocols for acute toxicity and bioaccumulation tests for both marine and freshwater sediments by the end of FY93. Also, as a result of this workshop, EPA will develop two other methods documents: 1) one on sediment spiking; and 2) one on sediment collection, handling, and storage. Parts of the document on collection, handling, and storage methods are already under development for a QA/QC guidance document which will supplement both the Inland and Ocean Testing Manuals for disposal of dredged material. This workshop also served to identify other research needs for assessment and management of contaminated sediments. Dr. Southerland reiterated that once the standard sediment testing methods are available, they can be used: immediately in the Superfund Contract Lab Program and in EPA Regional Environmental Services Divisions; EPA's Office of Pollution, Prevention, and Toxic Substances may begin a test rule process leading to publication of the method in the Federal Register; EPA's Office of Pesticide Programs may begin their Science Advisory Panel review process; and ASTM may begin their balloting process leading to completion of a standard method.

Dr. Ankley described the results of the freshwater breakout session. He stated that the bases for species selection for standardization were: current and historical acceptance; logistical considerations; and the availability of test methods. Dr. Ankley identified some of the major differences between freshwater and marine tests: 1) freshwater organisms are cultured and organisms are field collected for marine tests; 2) freshwater organisms are smaller and younger; and 3) freshwater organisms are generally epibenthic and marine test organisms are generally infaunal. Freshwater test conditions are quite sensitive to organic carbon and sediment oxygen demand, ammonia concentration and overlying water buffering capacity. The water column is more stable and constant in the marine environment. Dr. Ankley described performance based culture criteria for H. azteca. Consensus from the breakout session was reached on what criteria must be considered and what criteria should be considered. Factors that must be considered are: reference toxicants for short-term water only exposures, and control survival. Factors that should be considered are: parental survival; routine chemistry; food quality audit; routine culture renewal; time to emergence; and larval weight at different Instars. The same factors must or should be considered for C. tentans and C. riparius which will be incorporated into one culturing guidance document. Culture criteria for L. variegatus that should be considered are population doubling and routine chemistry. Reference toxicants must be considered for short-term water only exposures. The conclusion for culturing requirements for freshwater organisms was that no hard and fast guidelines would be developed but recommendations would be based on survey results discussed at this workshop, best professional judgement, and targeted research. The research study plan in Section II of this document, "Developing Guidance for Whole Sediment Toxicity and Bioaccumulation Tests with Freshwater Invertebrates" describes the tasks to be performed in developing the final protocols.

Dr. Ankley also summarized major issues discussed concerning the freshwater toxicity tests. The following major issues related to the use of H. azteca were covered in the session:

1) Age of test animals.

There appears to be a range of ages most appropriate for testing. Organisms of age 0-14 days are most appropriate. It may be best to use animals of age 7-14 days, but difficulties in recovering the animals may be encountered.

2) Length of test.

The length of the test agreed upon was 10 days with survival as the endpoint.

3) Feeding.

A minimal amount of food is necessary, but more data on feeding must be generated.

4) Water renewal.

Limited renewal of test water was recommended (1 volume/day).

5) Sediment volumes.

Sediment volume in the 1/2 liter range was recommended but it seems acceptable to use smaller test volumes.

6) Grain size.

There does not seem to be a grain size effect in the short term Hyalella test.

7) Strains of animals.

There are different strains of Hyalella used for testing. Reference toxicant comparisons of the strains are needed.

In discussing the Chironomus test it was agreed that minimal feeding would be appropriate (the acceptability of a change in total sediment organic carbon during the test of .01% - 1.0% was discussed). Workshop participants did not believe that genetic diversity was an important issue for Chironomus.

In discussion of the Lumbriculus bioaccumulation test a number of conclusions were reached:

1) The protocol should recommend a 28 day test.
2) No feeding is needed during the test.
3) The test can be conducted with water renewal or static conditions.
4) Standard lipid content should be addressed in the document.
5) The issue of sediment avoidance by Lumbriculus may be important.
6) Rigorous techniques have been developed to purge the gut in clean water. The contribution of the gut may be negligible. Research is needed to see if purging is necessary.

Dr. Swartz presented the results of the marine breakout session. It was decided that issues related to the sediment toxicity tests themselves should be separated from issues related to sediment manipulation and handling. The ASTM process for standardizing methods will be used to allow for peer review and more general participation in the process. Continued EPA Program Office and Regional involvement in test method development is needed. Focus was placed on two classes of concern: issues upon which consensus can be reached, and issues that needed research. R. abronius is the farthest advanced test species and it was determined that there were no critical research needs. The following consensus issues were identified for R. abronius: 1) a written document can now be developed; 2) consensus can be reached on the appropriate reference toxicant test; 3) relative sensitivity of the test can be documented with existing data; 4) adequate data on seasonality exist; 5) shipping and handling needs can be identified with existing data; and 6) waste disposal and safety protocols can be addressed. Research issues identified for A. abdita were: grain size tolerance; sensitivity of ovigerous females; and interpretation of control survival (many labs have had difficulty reaching 80-90% survival); additional field validation; and seasonal sensitivity. Research issues identified for L. plumulosus were: effects of salinity on test results; acclimation of animals; different test results due to differences in culturing methods, acclimation and salinity; sensitivity to ammonia and PAHs; interspecies comparisons; and sensitivity differences between laboratory and field collected species. Dr. Swartz noted that this species has only been tested during the past 2-3 years. Research needs identified for E. estuarius were: the effects of light on sensitivity; sensitivity to reference toxicants; interlaboratory comparison; and field validation. Dr. Swartz also summarized the discussion on Lepidactylus, a sensitive species that shows promise for use in sediment toxicity testing. It is similar in sensitivity to Eohaustorius, and has grain size sensitivity similar to Rhepoxynius. A major validation study conducted by Environment Canada was also described. Canadian draft manuals on sediment collection, handling, manipulation and spiking will provide a basis for standardization of these techniques.

Dr, Scott discussed the next steps for development of chronic tests which were judged to be at least 2 years behind acute methods. Leptocheirus and Ampelisca were identified as the 2 most promising species for the chronic tests. Technical issues that must be addressed for chronic test development are: standardization of the age of the species to be used; species sensitivity; nutritional requirements; feeding protocols; sediment aging; and selecting proper endpoints and biological responses. A number of endpoints for chronic test were discussed and are described in the breakout session notes.

Dr. Lee summarized the discussion of the marine bioaccumulation tests. Several approaches to bioaccumulation testing were discussed. These included: use of equilibrium partitioning models; kinetic modeling; and the direct approach of testing field collected sediment. The development of tissue residue criteria for the protection of human health was identified as a research need. It was agreed in the breakout session that the existing 28 day protocols for Neries and Macoma would be standardized. Field validation and round robin testing will be required to complete development of this protocol. Over a longer period of time, it will be necessary to develop kinetic models. It will also be necessary to develop guidance on the ecological significance of tissue residue levels.

Dr. Southerland concluded the meeting by thanking all the participants for their hard work and reminded everyone that methods protocols would be completed for the species discussed by each workgroup by the end of fiscal year 1993 (October 1, 1993). The following fiscal year, if more funds become available, the focus will be on standard chronic test methods and sediment toxicity identification evaluations.

WORKPLANS

The following workplans describe research that will be undertaken to develop sediment toxicity test protocols for marine, estuarine, and freshwater organisms. Funding has been provided to complete work on test protocols for species included in the workplans during fiscal year 1993. Work to develop additional test protocols will be completed if additional funding is available. The workplans were developed on the basis of discussions held at this workshop.

Technical Approach

This workplan was developed on the basis of discussions held at an EPA Office of Science and Technology (OST)/Office of Research and Development (ORD) sponsored workshop on the standardization of sediment toxicity tests. Meetings were also held with ORD scientists from the Environmental Research Laboratories in Narragansett, Rhode Island, and Newport, Oregon, to discuss and identify the objectives of the proposed work. In addition, some preliminary analyses of the statistical characteristics of acute sediment toxicity data were conducted.

This workplan describes research to be conducted to standardize acute, chronic, and bioaccumulation tests for sediment toxicity. The standardization of these methods is critical because of the need to instill consistency in their application in Federal, Regional and State sediment characterization programs. This consistency is of particular importance to the implementation of EPA's Contaminated Sediment Management Strategy. The strategy calls for completion of a national inventory of contaminated sites and continued monitoring to assess the extent and severity of the problem. These actions will require a nationally consistent means for determining sediment quality.

The general approach to standardization of sediment bioassays is to start with reasonably well-defined procedures, such as the ten-day sediment bioassay with the amphipod Rhepoxynius abronius, and proceed to more complicated, less well-defined procedures, for example, chronic tests and bioaccumulation tests. Field validation of test procedures completes the cycle of standardization prior to implementation of the methods.

The research described in this work plan supports the development of standard methods for acute and chronic bioassays and bioaccumulation tests. This work will concentrate on development to the ASTM "Standard Test Method" phase of the ten-day acute bioassay procedure with four species of amphipods. Other activities will support the development of the chronic bioassay and the bioaccumulation test to the ASTM "Standard Guide" phase. The latter documents can benefit from the ASTM review process, without the rigorous scrutiny necessary for standard methods.

Standard protocols for acute sediment toxicity tests using the benthic marine and estuarine amphipods Ampelisca abdita, Rhepoxynius abronius, Leptocheirus plumulosus, and Eohaustorius estuarius will be prepared. The protocols will include details on: culture and/or acquisition of test organisms; test design; QA/QC requirements; effects of abiotic factors; contaminant interactions; reference sediment requirements; relative sensitivity; biological significance of acute toxicity; and interpretive guidance. Additionally, protocols for the conduct of the 28-day bioaccumulation test, and for the collection, handling, and spiking of test sediments will be prepared.

The approach to completion of each research task is presented below. Research will be conducted at the SAIC, Inc. in Narragansett, RI, testing center in consultation with EPA's Environmental Research Laboratories in Narragansett, RI, and Newport, OR, the EPA Headquarters Office of Science and Technology, and EPA's Tiered Testing Workgroup.

Specific Research Tasks and Methodological Approach

Research to Support the Acute Standard Method:

There are a number of technical areas where data are needed to support an understanding of the responses of the four amphipod species to sediment contaminants. Some of the factors that affect an organism's response to contaminants are temperature, salinity, and grain size. Other sediment characteristics not related to contaminants, e.g., ammonia, sulfides, and low dissolved oxygen, may cause toxicity thus confounding the interpretation of contaminant effects. This workplan focuses on elaborating test species responses to salinity, ammonia, and grain size where existing data are lacking. Temperature effects are not important since a standard species-specific temperature within the upper tolerance range is normally chosen. Similarly, low dissolved oxygen or sulfide toxicity will not be addressed because the test chambers are well aerated during sediment exposures.

There also are no data on the relative sensitivity among these species to dissolved and sediment-associated contaminants. Field validation data also are lacking for three of the four species. In addition to the collection of supplementary data through the conduct of laboratory experiments, existing data on these elements of the tests will be gathered and synthesized.

The experimental design addressing each of the issues discussed below will be prepared and agreed upon in consultation with technical experts from the ORD's Environmental Research Laboratories and EPA's Office of Science and Technology.

Grain Size Tolerance:

Most marine and estuarine organisms prefer a particular range of sediment type that may be related to feeding or burrowing habits. In contaminant effects studies, sensitivity to particle size is important since unusual stress associated with extremes of the particle size range may induce mortality. The effect of fine particle size as an artifact causing non-contaminant induced mortality in Rhepoxynius abronius is well understood, and algorithms describing that relationship have been developed. It is possible that the opposite effect, i.e., coarse particle size-induced mortality, may occur in Ampelisca abdita. While contaminants are generally associated with fine sediments, contaminated coarse sediments can be extremely toxic also. Experiments will be conducted to identify the potential for grain size effects in both Ampelisca and Leptocheirus.

The hypothesis to be tested is that extremes in sediment particle size do not cause excessive mortality in the test organisms. To test this hypothesis, a range of particle size treatments (up to five) will be established by mixing, by volume, non-toxic coarse sediments with silt/clay sediments. Natural sediments with different particle sizes may also be tested. Each species will be exposed to these sediment mixtures using the existing ASTM procedure for the conduct of 10-day solid phase bioassays.

Existing data on Ampelisca's response to coarse particle sizes will also be reviewed and analyzed. This analysis will test the hypothesis that coarse particle size sediments do not cause excessive mortality any more frequently than do fine particle size sediments. The existing data base includes over 800 sediments tested in the EPA's Environmental Monitoring and Assessment (EMAP) and NOAA Status and Trends program. An extensive data base on Leptocheirus does not exist at present.

Ammonia Tolerance:

Sediment-associated ammonia has been suspected of causing toxicity in amphipods during the conduct of sediment toxicity tests when the tests are conducted under static conditions. Recent work with Ampelisca has established its tolerance limits to ammonia. Experiments will be conducted to establish the concentrations of ammonia in sediment pore water that have the potential to cause toxicity to the other three test species in 10-day static exposures.

Three sets of experiments will be conducted with Rhepoxynius, Leptocheirus, Ampelisca and Eohaustorius. In the first, a water-only LC50 will be derived through the conduct of a ten-day amphipod exposure to ammonia-spiked water. In the second experiment, an LC50 and NOEC will be derived for overlying water, by exposing each species in a 10-day solid phase bioassay to ammonia-spiked overlying water. Finally, LC50s and NOECs will be derived for sediment-spiked ammonia.

These tests will not only identify the ammonia concentrations where toxicity might be expected, but also the relative toxicity of each species to pore water and overlying water ammonia concentrations will be determined.

Salinity Tolerance:

It is important to establish the range of salinities over which test organisms can be exposed in order to avoid inducing toxicity due to salinity stress alone. The salinity ranges for Eohaustorius and Rhepoxynius are known to be broad and narrow respectively. The ranges for Leptocheirus and Ampelisca will be determined. The salinity range from 0-35 ppt will be tested in 5 ppt intervals using 10-day exposures. The sediments will be acclimated to the test salinity but the amphipods will not. The laboratory data will be evaluated in light of existing information on the distribution of these two species relative to salinity.

This research will not address the potential interactive affects of sublethal salinity stress and contaminant toxicity within the non-lethal salinity range.

Relative Sensitivity:

Typically, only one of the four amphipod test species will be used in a solid phase assessment of sediment toxicity. The species of choice would be selected for a number of reasons including: availability; regional interest; and particle size compatibility. The interpretation of a toxic response will require an understanding of the sensitivity of the test organism relative to that of "benchmark" species for which a large data base exists. Large data bases do exist for Rhepoxynius and Ampelisca, however, direct comparisons of the two species are rare.

Experiments will be conducted to test the hypothesis that there are no differences in sensitivity of the four amphipod species. The relative sensitivity to two inorganic and two organic compounds will be determined through two sets of experiments with the four species: four-day water-only and 10-day spiked sediment bioassays. LC50s for each species and exposure type will be determined. The relative sensitivity to field sediments of known contaminant concentrations and toxicity will also be determined in round robin testing described below. Further information will be gained by analysis of existing data on the relative sensitivity of these species.

Field Validation:

The ultimate utility of these acute bioassay methods will depend on the extent to which they predict contaminant effects in natural populations and communities. The toxic response in Rhepoxynius has been related to the absence of amphipods and alterations in benthic community structure.

A field validation of the acute response is necessary for Ampelisca, Eohaustorius, and Leptocheirus. Data are currently available from the EMAP and NOAA Status and Trends sites on the benthic community composition and abundance of amphipods. Concurrent data are also available on Ampelisca toxicity for many of these sites. These data will be reviewed and analyzed to determine if sufficient data exist to field validate the acute test for this species. The criterion for validation will be the co-occurrence of toxicity with decreased amphipod abundance and/or significant benthic community effects.

Analysis of these data will identify sites that may be used to validate the test for Leptocheirus. Additional samples, not to exceed 20, will be collected in cooperation with these programs for toxicity tests with this species. Similarly, if the data on Ampelisca are insufficient, this species will also be tested.

Testing Summary:

Grain size
- o Ampelisca - 10-day sediment - 5 treatment levels - 2 runs
- o Leptocheirus - 10-day sediment - 5 treatment levels -2 runs

Ammonia
- o Rhepoxynius - 4-day water - 5 treatment levels
  - 10-day sediment - spiked water - 5 treatment levels
  - 10-day spiked sediment - 5 treatment levels
- o Leptocheirus - 4-day water - 5 treatment levels
  - 10-day sediment - spiked water - 5 treatment levels
  - 10-day spiked sediment - 5 treatment levels
- o Eohaustorius - 4-day water - 5 treatment levels
  - 10-day sediment - spiked water - 5 treatment levels
  - 10-day spiked sediment - 5 treatment levels

Salinity tolerance
- o Ampelisca - 10-day sediment - 8 treatment levels
- o Leptocheirus - 10-day sediment - 8 treatment levels

Relative sensitivity
- o Four species - 4-day water - 5 treatment levels - 4 compounds
- o Four species - 10-day sediment - 5 treatment levels - 2 compounds

Field Validation
- o Ampelisca - 10-day - 20 sediments
- o Leptocheirus - 10-day - 20 sediments

At least 5 replicates will be used per run.

Research to Support the Chronic Standard Method:

Research will be initiated to develop standard methods for chronic bioassays with Leptocheirus and Ampelisca. Methods for the use of the former species are currently under development and validation by the EPA's Environmental Research Laboratory in Newport, Oregon (ERL-Newport) and others. Some of the research conducted to develop acute test methods will apply to the chronic methods as well. The EPA's Environmental Research Laboratory in Narragansett, Rhode Island (ERL-Narragansett) is also supporting research on chronic sediment bioassays with Ampelisca. Additional research with these two species will be defined at a later date when the chronic research plans for the ERL-Newport and Narragansett are prepared. Two to three 28-day chronic tests with up to five treatments each will be conducted. These experiments will focus on Ampelisca and will be designed based on the results of ongoing work at the two EPA ERL laboratories.

Since the ability to culture test species will be a requirement of chronic methods, additional research will address the culture requirements of Ampelisca. Short-term experiments will investigate the effects of culture container size and configuration, and feeding procedures and frequency on the size and fecundity of laboratory populations.

Research to Support the Bioaccumulation Standard Method:

The accuracy of the marine sediment bioaccumulation test is poorly quantified, and the extent to which it predicts tissue residues under field conditions is unknown. The accuracy, as defined by a field validation of the laboratory results, is critical to the future development and application of this test. A key to the laboratory to field comparison is the achievement of steady state conditions in the laboratory exposures. Also, ideal circumstances would dictate that the site chosen be inhabited by the same organisms being used in the bioaccumulation test.

Research described in this workplan will provide an initial field validation of the bioaccumulation method using Macoma nasuta and Neries virens. This research entails a minimum of a 60-day laboratory exposure to a naturally contaminated sediment with measurements of tissue residues in each species at a minimum of two time intervals (28 and 60 days). These values will be compared to those measured in field collected organisms, preferably the same species, inhabiting the same sediments.

Up to 50 tissue residue and eight sediment residue measurements will be required for this research. Chemical analyses will target high and low molecular weight PAHs, metals, and chlorinated compounds.

Criteria for the selection of an appropriate site include the presence of the test organisms, or an adequate sediment-ingesting surrogate, and concentrations of target contaminants sufficiently high to be bioaccumulated, but not high enough to cause mortality in test organisms. Only one site validation will be conducted due to the limited availability of resources. Therefore, the site will be carefully selected after consultation with ORD and OST experts. The same consultation will be required to finalize the experimental design.

Statistical Analysis of Acute Bioassay Data:

The currently accepted criterion for the assignment of toxicity to a sediment sample relies on the detection of a statistically significant decrease in survival in a test sediment relative to that in a control (or reference) sediment. This research will provide a rigorous analysis of acute test data for the four species to determine a level of statistical significance that relies on the variability of the test performance among and between test runs. The goal of these analyses is to describe procedures for examining the statistical nature of acute toxicity data bases relative to variations in control mortality, test precision, and the ability to determine minimum detectable differences. Preliminary analyses of selected Ampelisca data indicate that minimum detectable differences may range from 20 to 30%. Ultimately, these statistical differences can be compared to differences in mortality that are considered biologically significant.

The most complete data sets for these analyses are from tests using Rhepoxynius and Ampelisca. Statistical procedures for the determination of minimum detectable Differences will be applied to subsets of these data that will account for intra- and interlaboratory variability. Analyses will also be conducted on control data to determine its statistical distribution and the need, if any, for data transformation.

Acute Round Robin Tests:

Round robin testing will be required in order to determine the extent of interlaboratory variation in the acute sediment bioassays and to assess logistical constraints (e.g., shipping effects) associated with each species. Although interlaboratory test data are available for both Rhepoxynius and Eohaustorius, it will be important to include them in any round robin conducted to provide direct comparison with the other two species.

Four laboratories will be evaluated in this task. Up to four naturally contaminated and clean sediments will be tested with each of the four species by each laboratory. In addition, an LC50 will be determined for a highly contaminated natural sediment. Test organisms will be provided to each laboratory by the same supplier, and each species may be provided by a different supplier. Tests will be conducted according to draft standard methods described below.

Preparation of Draft Standard Methods:

Draft standard methods will be prepared for acute sediment bioassays with the four species of amphipods discussed above. These methods will be based on the existing ASTM method for these tests, as modified with the data generated from experiments described in this workplan. With the exception of the Rhepoxynius document which is based on much sediment bioassay experience, data analyses and syntheses for the remaining three species will be required. These analyses will include an assessment of the control response, summary of existing data, statistical power, habitat preference, and known tolerances of non-contaminant factors.

The ASTM format will be followed for this and all other methods developed under this workplan. In this format, the documents will be submitted for review and approval under the ASTM Subcommittee E47.03 sediment toxicology balloting system.

A draft guide will be prepared for chronic sediment bioassays using Leptocheirus. This document will be based on the work and procedures of ERL-Newport and the state of Maryland. Again, data generated from experiments described in this workplan, or by other groups, will be included in the draft guides.

Two draft guides for the 28-day bioaccumulation test with Macoma and Nereis will be prepared. These documents will be based on the existing EPA method for the former species. That EPA method also is a draft ASTM guide.

Additional standard methods for sediment collection, handling, and spiking will be prepared in the ASTM format. The sediment spiking procedure will follow those developed by the ERL-Newport laboratory for the sediment quality criteria program. The sediment collection and handling document will be based on an existing guide prepared by ASTM. This document will be revised in conjunction with ongoing efforts to develop standard methods for freshwater sediment bioassays. Specifically, a committee of experts will be formed to come to consensus on key issues. This group will meet at the fall 1992 and spring 1993 ASTM meetings to discuss the proposed revisions to the existing document.

Schedule

Acute Testing:

Complete ammonia tolerance tests with Rhepoxynius abronius, Leptocheirus plumulosus, and Eohaustorius estuarius - data report **(Jan/93)**

Complete grain size tolerance tests with Ampelisca abdita and Leptocheirus plumulosus - data report **(Jan/93)**

Complete salinity tolerance tests - data report **(Jan/93)**

Complete comparative sensitivity tests with spiked sediments - data report **(Mar/93)**

Complete field validation data analysis and testing - data report **(Sept/93)**

Chronic Testing:

Complete development of culture methods for Ampelisca - draft protocol **(Sept/93)**

Bioaccumulation Tests:

Complete laboratory testing and chemical analysis for field validation - data report **(Sept/93)**

Statistical Analysis Procedures:

Completion of statistical analyses for minimum detectable difference for each of the four species - data report **(Jun/93)**

Acute Protocol Round Robin:

Completion of acute test round robin - data report **(July/93)**

Document Preparation:

Draft Standard Method for Rhepoxynius abronius Acute Test **(Apr/93)**

Draft Standard Method for Ampelisca abdita Acute Test **(Sept/93)**

Draft Standard Method for Leptocheirus plumulosus Acute Test **(Sept/93)**

Draft Standard Method for Eohaustorius estuarius Acute Test **(Sept/93)**

Draft Guide for Macoma nasuta Bioaccumulation Test **(Sept/93)**

Draft Guide for Neries virens Bioaccumulation Test **(Sept/93)**

Draft Guide for Leptocheirus plumulosus Chronic Test **(Sept/93)**

Draft Standard Method for Sediment Spiking **(Apr/93)**

Draft Standard Method for Sediment Collection, Handling, and Storage **(Sept/93)**

Development of Sediment Toxicity Bioassays for Freshwater Organisms

Technical Approach

This workplan was developed on the basis of discussions held at an EPA sponsored workshop on standardization of sediment toxicity tests. Determining the significance of contaminants in sediments to aquatic organisms is a challenging new area in environmental toxicology. Mounting evidence exists of environmental degradation in areas where water quality criteria are not exceeded, yet organisms are adversely affected. Historically, emphasis has been placed on evaluating contaminant effects in surface waters, not sediment. Most assessments of water quality focus on water-soluble compounds and sediment is considered a safe repository of sorbed contaminants. This approach emphasizes testing organisms in the water column without considering the fate of chemicals in sediment.

A variety of methods have been developed to evaluate sediment contamination. These procedures range in complexity from short-term tests measuring effects of individual contaminants on single species to long-term tests that determine effects of chemical mixtures on the function of microcosms. The sediment phase evaluated includes whole sediment (often referred to as the solid phase), suspended sediment, elutriates, and sediment extracts. The amount of sediment tested ranges from a few grams to over 800 liters. The organisms tested include algae, macrophytes, fish, and benthic, epibenthic, and pelagic invertebrates.

Ideally, a sediment test should be rapid, simple, and inexpensive if the objective of the study is to screen a large number of samples in a timely manner. Identification of severely contaminated sediment can be accomplished with existing methods. However, concentrations of chemicals in sediments that are not acutely lethal may interfere with the ability of an animal to develop, grow, or reproduce. Information concerning chronic effects and long-term bioaccumulation of chemicals from sediment is needed to identify moderately contaminated areas and to understand the environmental significance of these contaminants. Most estimations of chronic sediment contaminant effects have been based on 7- to 14-d exposures with midges, amphipods, polychaetes, or cladocerans. However, the partial life-cycle exposures may not always include the most sensitive life stages of the test species. Testing sensitive life stages in long-term exposures may provide a better measure of sublethal chemical toxicity.

Natural physical properties such as sediment texture may influence the response of animals in whole sediment tests. Research is needed to determine the influence of "non-contaminant" factors such as sediment particle size, organic content, and water quality on the response of test animals. This information is needed to distinguish responses to contaminants from responses to natural sediment characteristics.

Direct comparisons of animals exposed in the laboratory and in the field are required to verify results from laboratory testing procedures. The assumption that laboratory results for a specific sediment represent effects of similar sediments in the field needs to be evaluated. Hazard evaluation of contaminated field sediments that integrate data from laboratory exposures, chemical analyses, and *in situ* field assessments provide strong complementary evidence of the degree of pollution-induced degradation to aquatic and benthic communities.

The goal of this research project is to develop state-of-the art, standardized protocols for assessing the potential effects of contaminated sediments on aquatic ecosystems. These laboratory tests are an essential component to the tiered testing approach currently being developed by EPA. The general strategy behind the research is to start with the standardization of reasonably well-defined test procedures (10-d acute toxicity tests with benthic invertebrates), proceeding to less well-defined protocols (bioaccumulation tests, food chain models, chronic toxicity tests, toxicity identification evaluation), and ultimately culminating in field validation of the tests. Because many contaminants of concern in sediments bioaccumulate, this research will emphasize development and validation of toxicity and bioaccumulation tests to residue-effect endpoints based on tissue concentrations. Part of this effort will involve developing toxicokinetic and metabolism models for species exposed to different classes of representative sediment contaminants. This information is needed in order to develop realistic models for predicting exposure of organisms through the food chain, and will also provide a technical basis for assessing the use of risk based residue-effect models.

The following objectives (elements) and associated timelines are based on the assumption that support for this research effort will be available at comparable levels for 3 to 5 years. Available FY92 and FY93 funding will support only research activities dealing with Objectives 1, 2, and 3. Research will be conducted at the U.S. Fish and Wildlife Service National Fisheries Contaminant Research Center in Columbia, Missouri (NFCR-C) and EPA's Environmental Research Laboratory in Duluth, Minnesota (ERL-Duluth) in consultation with an established workgroup of experts on freshwater sediment toxicity testing, EPA's Office of Science and Technology, and an EPA Tiered Testing Workgroup.

Specific Research Objectives

1. Standard Protocol for an Acute Toxicity Test with Hyalella azteca (FY93)
2. Standard Protocol for an Acute Toxicity Test with Chironomus tentans (FY93)
3. Standard Protocol for a Bioaccumulation Test with Lumbriculus variegatus (FY93)
4. Toxicity Identification Evaluation (TIE) Procedures for Contaminated Sediments (FY94)
5. Standard Protocol for a Chronic Toxicity Test with Hyalella azteca (FY94)
6. Standard Protocol for a Chronic Toxicity Test with Chironomus tentans (FY95)
7. Develop a Generalized Model for Predicting the Metabolism of Common Sediment-Associated Contaminants in Benthos and Fish (FY95)
8. Develop an Effects-Based Tissue Residue Model for Assessing the Risk of Sediment-Associated Contaminants (FY96)
9. Summarize Field Validation Studies for Standardized Toxicity and Bioaccumulation Tests for Freshwater Sediments (FY96)

Experimental Design and Methodological Approach

Investigations of sediment toxicity and bioaccumulation are limited by a lack of understanding of the factors controlling contaminant availability in sediment. Additionally, a lack of available standardized methods also limits the use of sediment tests in contamination assessments. ASTM Subcommittee E47.03 on Sediment Toxicology has developed guides for assessing the bioavailability of contaminants associated with sediments (e.g., ASTM E 1383-92 "Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates"). These guides are used to evaluate the toxicological hazard of contaminated sediment, soil, sludge, drilling fluids, and similar materials. The Subcommittee developed general guides and not standard test methods or protocols, because most procedures for evaluating contaminated sediment have only been recently developed. Definitive protocols are needed which describe specific test methods.

Objective 1: Standard Protocol for an Acute Toxicity Test with Hyalella azteca (FY93)

The protocol will include details for culturing and testing the amphipod, including test system design. Also covered will be the development of a standard reference sediment, procedures for reference toxicants, procedures for interpreting the effects of abiotic factors (e.g., particle size) on test results, use of cell lines for screening complex hydrophobic compounds and determining the potency of these compounds to aquatic organisms, the results of a relative sensitivity analysis (from a series of single chemical tests) for H. azteca, evaluation of genetic variability in laboratory cultures of the amphipod, and results of preliminary round-robin studies.

Hyalella azteca will be cultured to produce known-age or known-size animals. NFCR-C will culture amphipods using SOPs supplied by ERL-Duluth. ERL-Duluth recommends a diet of diatoms and YCT. NFCR-C recommends a diet of Tetramin and maple leaves. Performance of cultures will be compared using known-age or mixed-age methods and various diets.

A standard control sediment will be developed for use in determining the acceptability of the test and will facilitate inter-laboratory comparisons. A particle size distribution and concentration of total organic carbon will be selected to be representative of freshwater sediments. NFCR-C will evaluate KCl and ERL-Duluth will evaluate $CuSO_4$ for use as a reference toxicant.

NFCR-C will establish a culture of the ERL-Duluth strain of Hyalella azteca. Relative sensitivity of this strain will be compared to the NFCR-C strain. Taxonomy of both strains will be confirmed by an identified expert.

Preliminary round robin studies will be conducted by 8 laboratories using water only exposures (Phase 1) and whole sediment exposures (Phase 2):

a. Phase 1: Water only, 4-d exposure, 3 species (e.g., common strain), 1 reference toxicant (KCl).

b. Phase 2: Whole sediment, 10-d toxicity exposures, 3 species (e.g., common strain), 2 sediments (medium and high toxicity) + control. Versions of ERL-Duluth methods would be used.

c. Timeline:
   1. Identify methods and laboratories for testing
   2. Phase 1 testing: October, 1992
   3. Phase 2 testing: February, 1993

Objective 2: Standard Protocol for an Acute Toxicity Test with Chironomus tentans (FY93)

The protocol will include details for culturing and testing C. tentans, including test system design. Also covered will be the development of a standard reference sediment, procedures for reference toxicants, procedures for interpreting the effects of abiotic factors (e.g., particle size) on test results, use of cell lines for screening complex hydrophobic compounds and determining the potency of these compounds to aquatic organisms, the results of a relative sensitivity analysis (from a series of single chemical tests) for C. tentans, and results of preliminary round robin studies. Preliminary round robin studies will be conducted by 8 laboratories using water only exposures (Phase 1) and whole sediment exposures (Phase 2).

Objective 3: Standard Protocol for a Bioaccumulation Test with Lumbriculus variegatus (FY93)

The oligochaete Lumbriculus variegatus is the most promising benthic test species available for a standardized freshwater bioaccumulation test. Research will be focused on field validation of bioaccumulation tests with Lumbriculus, as well as analysis of the kinetics of bioaccumulation of different classes of chemicals of concern. Lumbriculus variegatus will be cultured at NFCR-C. ERL-Duluth will supply their SOPs and animals. NFCR-C will determine performance of the cultures using reference toxicants and estimates of population dynamics.

Specific Research Tasks to Develop Acute Toxicity and Bioaccumulation Test Protocols

Specific research tasks that must be completed to develop the acute toxicity and bioaccumulation test protocols are identified below. Each area of research is identified with a group or groups having primary responsibility for conducting the work: (Columbia = NFCR-C, WSU = Wright State University, Athens = NFCR-C Athens Georgia Field Research Station). An area of research designated "round robin" will be evaluated by laboratories participating in the round robin tests. An "*" indicates areas of research that will not be completely resolved by the end of fiscal year 1993. Best professional judgment will be used in some instances to make decisions regarding areas of research indicated with an "*". An area of research designated with an "@" was identified as a high priority research need at EPA's test method standardization workshop. NFCR-C will be responsible for writing the final document describing the standard protocols. Chemical analyses will be done by the U.S. Fish and Wildlife Service Patuxent Analytical Control Facility. This laboratory will meet QA/QC requirements and anticipate a 30 to 90 day turn around time for samples.

1. CULTURING

a. amphipods: known-age vs. mixed cultures

1. evaluate sensitivity to suite of compounds with different modes of action (Columbia, Athens, @)

2. consistency in size of organisms between known-age and mixed cultures (Columbia, WSU, @)

b. use of reconstituted water (*)

c. diet (*)

d. performance criteria (WSU, *, @)

2. WHOLE SEDIMENT TESTING

a. temperature: 20 to 25·C (*)

b. static renewal 1-4 volume additions/day (*)

c. methods for static renewal: evaluate water quality with various static renewal exposure systems (All)

d. chamber size: 30 mL to 1 L (typically 250 to 300 mL); volume of sediment: 100 mL (minimum for 300 mL chamber); sediment to water ratio between 1:1 and 1:4 (round robin, @)

e. known-age animals:

1. Hyalella azteca: 0 to 14 d old

a. evaluate using reference toxicants (All, @)

b. behavior in sediment by life stage (WSU, Columbia)

2. Chironomus tentans: 10 d old (*)

a. evaluate using reference toxicants (All, @)

3. Lumbriculus variegatus: adults (*)

a. evaluate using reference toxicants (All, @)

f. number of animals/chamber: minimum 10 (*)

g. number of replicates (chambers): power analyses are needed to determine desired number of replicates (Athens, @)

h. feeding (All, round robin, *, @):

1. Hyalella azteca: 6 mg rabbit pellets/MWF/20 animals (Columbia); 0.8 mg YTC/d (EPA); methods will be compared

2. Chironomus tentans: 4 mg Tetramin/d/10 animals

3. Lumbriculus variegatus: no feeding for bioaccumulation testing, 20 mg trout starter every 5 d/10 animals in toxicity testing

i. water quality: evaluate use of reconstituted water (*)

j. photoperiod: 16:8, minimum 25 foot candles (*)

k. endpoints: survival, growth (round robin, @)

l. test acceptability criterion (round robin, @)

3. WATER-ONLY TESTING

a. Reference toxicants: Water only, 4-d exposures, performed monthly. Columbia will evaluate KCl, WSU will evaluate $CdCl_2$, and ERL-Duluth will evaluate $CuSO_4$ (@).

b. Use of reconstituted water (All, @)

c. Use of phenol or another non-ionic organic as a reference toxicant for water-only or sediment testing (*)

4. STANDARD CONTROL SEDIMENT (@, Columbia, Athens)

5. PROCEDURES FOR INTERPRETING EFFECTS OF ABIOTIC FACTORS (Duluth, Columbia, WSU, @)

Reconstituted sediment will be used to evaluate particle size and organic carbon. ERL-Duluth is developing databases and regression equations with 50 sediment samples for all 3 species. ERL-Duluth is developing manuscripts dealing with interpreting the effects of ammonia. WSU is evaluating the influence of low dissolved oxygen.

6. RELATIVE SENSITIVITY ANALYSIS (Duluth, @)

ERL-Duluth is developing a database for 12 chemicals with all 3 species using 10-d flow-through water-only exposures.

7. GENETIC VARIABILITY OF Hyalella azteca (All, @)

Columbia and WSU have started cultures of the ERL-Duluth strain of Hyalella azteca. Relative sensitivity of strains to a suite of compounds with different modes of action will be evaluated.

8. PRELIMINARY ROUND-ROBIN STUDIES (WSU, @)

a. Toxicity testing: 8 laboratories

1. Phase 1: Water only, 4-d exposures, Hyalella azteca (Chironomus tentans next spring), KCl reference toxicant. October 1992.

2. Phase 2: Whole sediment, 10-d toxicity exposures, 2 sediments + control. Versions of ERL-Duluth methods used. February 1993.

9. EXPERIMENTAL DESIGN FOR FIELD VALIDATION OF BIOACCUMULATION TESTS WITH LUMBRICULUS

a. Objectives

1. Kinetics of bioaccumulation of different classes of chemicals of concern with Lumbriculus

2. Field validation of laboratory bioaccumulation exposures with Lumbriculus

b. Experimental design

1. Field-collected sediments (2 sediments)

(Select sediments with broad range of K_{ow} compounds (up to K_{ow} log 7-8); organic carbon/lipid normalize.

Suggested location for sediment collection:

a. Little Scioto River in Ohio: high PAHs and metals, possibly PCBs

b. Huntsville Alabama: DDT and metabolites

2. Field-collected oligochaetes (5 samples/sediment)

3. Laboratory-exposed oligochaetes (56-day exposure sample over time)

c. Field collection

1. Field-collected oligochaetes

5 samples x 2 sediments = 10 oligochaete samples

2. Field-collected sediment

A. 2 sediments: Little Scioto and 1 Huntsville

B. Collect multiple grabs of sediment (about 4 L/grab). Homogenize and split into two subsamples. one 2-L subsample for sediment chemistry and a second 2-L subsample for field-collected oligochaetes. Sieve oligochaetes in the field. Repeat 4 L grab sampling until enough biomass and sediment is collected. Ship oligochaetes and sediments to Columbia. No depuration of field-collected oligochaetes (comparison to 28-D sample w/o elimination).

d. Chemistry samples (metals and organics sampled from the same replicate)

1. Chemical-specific analyses:

A. Little Scioto River: PAHs, metals, possibly PCBs

B. Huntsville: DDT and metabolites

2. Sediment

2 replicates/sampling period
x 3 sample periods (day 0, 28, 56)
= 6 samples/sediment

3. Field-collected oligochaetes

5 replicates/sediment

4. Laboratory-exposed oligochaetes

A. Uptake: day 0, 2, 4, 7, 14, 28, 56

1. 3 replicates/sampling period
x 6 sampling periods (days 0, 2, 4, 7, 14, 56)
= 18 samples/sediment

2. 5 replicates/sampling period
x 1 sampling period (day 28)
= 5 samples/sediment

3. Total: 23 oligochaete samples/each sediment

B. Elimination: hour 12, 24, 48, 72, and day 7

1. 2 replicates/sampling period
x 5 sampling periods
x 2 treatments (with or without sediment)
= 20 samples/sediment

Additional Research Proposed for FY94-96

Objective 4: Toxicity Identification Evaluation (TIE) Procedures for Contaminated Sediments (FY94)

A draft document describing preliminary methods for sediment TIE has already been completed; further resources will enable completion of research focused upon issues such as pore water preparation, isolation and fractionation of high log K_{ow} non-ionic organics, species selection, TIE on whole sediments, and field validation.

Objective 5: Standard Protocol for a Chronic Toxicity Test with Hyalella azteca (FY94)

In addition to the research issues identified under Objective 1, a key factor to be addressed in the development of a chronic test would focus on appropriate toxicity endpoints. In addition, the length of the test will be evaluated; it may be, for example, that little additional information is gained in long-term tests as compared to short-term tests.

Objective 6: Standard Protocol for a Chronic Toxicity Test with Chironomus tentans (FY95)

See Objective 5.

Objective 7: Develop a Generalized Model for Predicting the Metabolism of Common Sediment-Associated Contaminants in Benthos and Fish (FY95)

Residue-based risk assessments for hydrophobic sediment-associated contaminants are highly dependent on accurate prediction and measurements of bioaccumulation. For super-hydrophobic chemicals, bioaccumulation potential can be significantly overestimated by models, which assume no metabolism, or by empirical exposure that are of insufficient length to account for the kinetics of biotransformation. Procedures will be developed whereby computer-assisted predictions of metabolic rates can be used to refine bioaccumulation estimates and identify associated uncertainties.

Objective 8: Develop an Effects-Based Tissue Residue Model for Assessing the Risk of Sediment-Associated Contaminants (FY96)

Current standard guides must be modified to provide a residue-effect based risk assessment approach for very hydrophobic chemicals. Studies must be longer in length and incorporate both dietary and water column routes of exposure to adequately quantify bioaccumulation. Toxicity endpoints must also include effects on reproduction and the probability of these effects must be related to residue levels. Research will be directed to provide improved test protocols and predictive models for assessing risk.

Schedule for Completion of Products in Fiscal Year 1993

The final product of work funded in FY93 will be standard protocols for acute toxicity tests with Hyalella azteca, Chironomus tentans, and the bioaccumulation protocol for Lumbriculus variegatus (Elements 1, 2, and 3). Draft protocols will be completed by September 30, 1993. Final protocols will be completed 90 days after receipt of comments from reviewers.

The ASTM format will be followed for all methods developed under this workplan. In this format, the documents will be submitted for review and approval under the ASTM Subcommittee E47.03 sediment toxicology balloting system.

OUTLINES
OF
SPEAKER PRESENTATIONS

EPA's Contaminated Sediment Management Strategy

Elizabeth Southerland, U.S. EPA Office of Science and Technology

In the 1980s EPA documented the extent and severity of contaminated sediment problems at sites throughout the U.S. Concerned with the mounting evidence of ecological and human health effects, EPA's Office of Water organized a Sediment Steering Committee chaired by the Assistant Administrator of Water and composed of senior managers in all the EPA offices with authority to handle contaminated sediments and EPA's ten Regional offices.

Over the past two years this committee has been preparing an Agencywide Contaminated Sediment Management Strategy to coordinate and focus EPA's resources on contaminated sediment problems. A draft outline of this strategy was released to the public this year to serve as a proposal for discussion in three national forums scheduled for April, May, and June. The draft strategy is designed around three major principles:

1. In-place sediment should be protected from contamination to ensure that the beneficial uses of the nation's surface waters are maintained for future generations;

2. Protection of in-place sediment should be achieved through pollution prevention and source controls;

3. Natural recovery is the preferred remedial technique. In-place sediment remediation will be limited to high risk sites where natural recovery will not occur in an acceptable time period and where the cleanup process will not cause greater problems than leaving the site alone.

The draft strategy includes several component strategies: assessment, prevention, remediation, dredged material management, research, and outreach. A brief summary of each of these elements follows.

In the assessment strategy EPA is committing to develop a national inventory of contaminated sediment sites and a pilot inventory of potential sources of sediment contamination, based on existing data. The two types of inventories will be complementary because the source database can be used to predict where sediments are contaminated in unsampled areas. The inventories will be designed so that EPA's prevention and remediation programs can use them to focus their resources on cleaning up the top priority sites and sources. Another key element of the assessment strategy is the commitment to develop a consistent; tiered testing strategy that will include a minimum set of sediment chemical criteria, bioassays, and bioaccumulation tests that all programs will agree to use in determining if sediment are contaminated.

The prevention strategy includes a variety of pollution prevention measures and source controls. The scale of contamination will guide the choice of a particular set of these measures. If a sediment contaminant is causing harm or risk at numerous sites nationwide, it may be

relatively inefficient to deal with the problem on a site-by-site basis. Instead, the strategy discusses nationally applicable responses, such as prohibitions or use restrictions under TSCA or FIFRA, technology-based effluent limitations for industrial dischargers, or a national initiative to revise water-quality based limits in NPDES permits. If atmospheric deposition appears to be a primary source of contamination, responses under the Clean Air Act will be considered. Where sediment contamination is a concern at particular sites, but not on a national scale, case-by-case assessments and response actions are recommended. Based on narrative and chemical-specific criteria and standards, EPA or a State can develop NPDES permit limits for discharges from industrial sources, municipal sewage treatment plants, stormwater outfalls, and combined sewer overflows. States that have nonpoint source control programs can take actions to reduce the contributions of these sources to sediment contamination.

EPA may remediate sediments under CERCLA, RCRA, CWA, and TSCA. The remediation programs will use the national inventory to assist in selecting sites for cleanup and the consistent tiered testing to assist in identifying contaminated areas and establishing cleanup goals. The remediation strategy emphasizes that sources of contamination should be controlled prior to remediation efforts unless the contaminated sediments pose a sufficiently great environmental hazard. In making remediation decisions, the strategy also points out that it is important to consider whether contaminated sediments at a site can be transported to downstream or offshore areas if left in place, thereby increasing the size of the contaminated area and making future remediation efforts much more difficult. Other factors to consider include the timeframe for natural recovery, the potential for contaminant mobilization during remediation, and the feasibility and cost of various treatment and removal options.

The maintenance of our nation's waterways for navigation requires the dredging and disposal of 250 to 450 million cubic yards of material each year. Dredged material testing manuals prepared jointly by EPA and the Corps of Engineers recommend the chemical and biological tests that should be conducted to determine if the material is contaminated and must be disposed of using special procedures. The tests selected for the Agencywide contaminated sediment strategy will be included in these dredged material testing manuals. The strategy also outlines additional guidance that will be developed by EPA and the Corps to improve the management of these materials.

The research strategy outlines all the work that EPA's Office of Research and Development (ORD) has planned on sediment chemical criteria, sediment bioassay and bioaccumulation test, fate and transport models, and remedial techniques. ORD is establishing a Resource Center to provide EPA offices with centralized technical assistance in evaluating sediment contamination and will also sponsor workshops and training sessions throughout the country.

The outreach strategy describes how EPA will work with other Federal agencies and State agencies to coordinate EPA's contaminated sediment activities with their efforts. EPA will strive to ensure that these agencies share sediment related research findings and innovative technologies. In addition, EPA is proposing a two-way public awareness program that will disseminate contaminated sediment information to the public and also incorporate information from the public into EPA activities.

GOALS OF OUR STRATEGY

- **Prevent Future Contamination of Sediments**
- **Manage Existing Sediment Contamination Using:**
  - **Pollution Prevention**
  - **Source Controls**
  - **Natural Recovery Where Appropriate**

GOALS (Cont.)

- **Remediate High-Risk Sites Where Natural Recovery Is Not Acceptable**
- **Ensure Environmentally Sound Management of Sediment Dredging and the Disposal of Dredged Materials**

PRINCIPLES OF OUR STRATEGY

- **Use Sound Science to Assess and Manage Sediment Contamination**
- **Assign Highest Priority to Activities with the Greatest Opportunity for Reducing Risks**
- **Continue to Develop and Improve Assessment Methods**
- **Conduct an Inventory and Improve Monitoring**

PRINCIPLES (Cont.)

- **Use Consistent Assessment Methods Across Programs**
- **Respond to Risks as Consistently as Is Possible**
- **Maintain Existing Sediment Quality through Pollution Prevention and Source Controls**
- **Implement Prevention and Control Measures, and Allow Natural Recovery as the Preferred Remedial Alternative**

PRINCIPLES (Cont.)

- **Assign Highest Priority to Remediating Contamination:**
  - **That Is Contributing to Substantial Risks**
  - **Where Delay Would Spread Harmful Contamination**
    - **Into Areas Where Remediation Is No Longer Feasible**
    - **Into Areas That Provide Critical Habitat**
  - **Where the Remedy Will Not Cause More Harm**
  - **Where the Agency Can Use Its Enforcement Authority**

PRINCIPLES (Cont.)

- **Costs Should Be Borne by Federal, State, and Local Governments and by Responsible Parties**
- **Build Alliances and Coordinate with Other State and Federal Agencies, with International Organizations, and with Private Parties**
- **Involve the Public through an Effective Outreach and Communications Program**

ELEMENTS OF OUR STRATEGY

I. Assessment
   - A. National Inventory
   - B. Consistent Tiered Testing
   - C. Monitoring

II. Research
   - A. Sediment Chemical Criteria
   - B. Bioassay/Bioaccumulation Methods
   - C. Fate and Effects Models
   - D. Remedial Technology Development/Demonstration
   - E. Technology Transfer

ELEMENTS (Cont.)

III. Prevention

A. Effluent Guidelines

B. Point Source Controls, Including CSOs and Stormwater

C. Nonpoint Source Controls

D. Review of Pesticides

E. Review of Toxic Chemicals

F. Additional Pollution Prevention Activities

ELEMENTS (Cont.)

IV. Remediation

- A. Enforcement-Based Remediation
- B. Superfund Cleanups
- C. RCRA Corrective Action
- D. PCB Cleanup Requirements
- E. CWA/Corps Remediation

V. Managing Dredged Materials

- A. Improved Testing and Management
- B. Applying Sediment Criteria
- C. Applying RCRA Criteria
- D. PCB Disposal Requirements

ASSESSMENT STRATEGY

I. National Inventory of Sites

- List Specific Geographic Areas and Potential Sources
- Rank High, Medium, Low Risk, or Known vs. Suspected Risk
- Better Estimate Extent and Severity of Problem
- Target Sites for Potential Pollution Prevention/Control Measures
- Target Sites for Potential Remediation

ASSESSMENT STRATEGY (Cont.)

II. Pilot Inventory of Sources

- List Specific Industries Using TRI, Effluent Guidelines Data, etc.
- Target Industries for Pollution Prevention/Controls

III. Agencywide Use of a Minimum Set of Tests

- Acute and Chronic Bioassays
- Chemical Criteria
- Bioaccumulation Tests/Models

ASSESSMENT STRATEGY (Cont.)

IV. Monitoring

- ORD's EMAP
- Monitoring Mission Statement
- Monitoring Task Force with USGS and Others
- Data System Modernization

RESEARCH STRATEGY

I. ORD Sediment Quality Research Initiative

- Chronic Toxicity Tests
- Improved Acute Tests
- Enhanced Bioaccumulation Tests
- Chemical Criteria
- Enhanced Fate and Transport Models
- Remedial Guidance and Technologies

II. Field Validation of Criteria and Bioassays

RESEARCH STRATEGY (Cont.)

III. ARCS Research and Demonstration Program

IV. Technology Transfer

- Consultation Center
- Public Workshops/Training Sessions

V. Research by Other Federal Agencies

- Corps—Bioassays, Risks of Upland Disposal
- Fish and Wildlife—Bioassays, Bioaccumulation

PREVENTION STRATEGY

I. Pollution Prevention

- Assess Risks of a Cluster of Persistent/Bioaccumulative Toxic Chemicals
- Ban or Restrict Use of Pesticides and Chemicals Causing Unreasonable Risks

II. Nonpoint Source Controls

- Use Section 319 Grant Set-Asides to Prevent Sediment Contamination
- Include in Agricultural Pollution Prevention Strategy

PREVENTION STRATEGY (Cont.)

III. Point Source Controls

- Consider Sediments When Regulating Industries with New or Revised Effluent Guidelines
- Learn How to Write Sediment Quality- and Bioconcentration-Based Permit Limits
- Write Permit Limits for High-Priority Dischargers
- Limit Discharges of Toxic Air Pollutants

REMEDIATION STRATEGY

I. Enforcement-Based Remediation

- Use CWA, CERCLA, RCRA, and TSCA to:
  - Compel Responsible Parties to Clean Up Sites
  - Recover Costs for EPA-Performed Cleanups
  - Coordinate with Natural Resource Trustees to Seek Restitution
- Coordinate with States Who Have Additional Authorities and Major Roles to Play

REMEDIATION STRATEGY (Cont.)

II. CERCLA Remediation

- **The Revised Hazard Ranking System Assigns Greater Weight for Sediment Contamination**
- **Consider Sites on National Inventory for Scoring under the HRS**
- **Use Agencywide Sediment Contamination Tests in RI/FS Stage of Remediation Process**

REMEDIATION STRATEGY (Cont.)

III. RCRA Remediation

- Use National Inventory to Target Facility Investigations
- Use Agencywide Sediment Contamination Tests in Hazardous Waste Facility Investigations

DREDGED MATERIAL MANAGEMENT STRATEGY

- Develop First National Guidance on Testing Dredged Material for Discharge into Fresh and Estuarine Waters
- Implement the Revised National Guidance on Testing Dredged Material for Discharge into Ocean Waters
- Develop a Document on the Environmental Factors to Consider When Evaluating Disposal Options
- Review PCB and RCRA Disposal Requirements Using Agencywide Remediation Principles

Standardized Sediment Testing: Needs and Requirements of Key Agency Programs

Thomas Armitage, U.S. EPA Office of Science and Technology

I. Office of Wetlands, Oceans, and Watersheds

A. Oceans and Coastal Protection Division

1. Regulatory Responsibilities:

a. Determine the acceptability of materials, including dredged materials, for ocean dumping under the Ocean Dumping Regulations.

B. Statutory Authority

1. Marine Protection, Research, and Sanctuaries Act (MPRSA);

2. Ocean Dumping Regulations (40 CFR 227 and 228)

C. Major Needs and Requirements for Standard Sediment Testing Methods:

1. Primary interest lies in testing dredged material to determine possible effects of disposal.

2. Methods for the following tests should be standardized: chemical analytical testing, toxicity bioassays, and bioaccumulation.

3. A sufficient number of test organism taxa must be standardized in order to provide an adequate range of test species and be applicable to a wide range of sites and contaminants.

a. It is recommended that standardized organisms to be used in acute toxicity tests be from the taxonomic order Amphipoda, and include at least the organisms Ampelisca abdita and Rhepoxynius abronius.

b. Standard organisms for bioaccumulation tests should include a number of species from the following taxonomic groups: polychaetes (particularly Neanthes sp, Neries sp, and Nepthys sp), molluscs (particularly Macoma sp), and crustaceans. Because of problems with availability and relative sensitivities, we need several organisms standardized from more than one taxonomic order.

4. Development of chronic sediment bioassays is needed for the Ocean Dumping Program; currently, standard whole sediment chronic bioassay tests are not available.

5. Standard methods for use in marine sediment chemistry analysis have been developed for the Clean Water Act 301(h) program (monitoring ocean outfalls of sewage treatment plants), and are the methods specified for use in testing sediment for ocean disposal. The Oceans and Coastal Protection Division suggests that these methods be adopted for marine sediments unless other methods can be standardized and used routinely.

D. Existing Program Guidance and Tests Used to Assess Sediment Contamination:

1. The testing manual, "Evaluation of Dredged Material Proposed for Ocean Disposal" outlines procedures for water column and benthic effects tests.

   a. The testing manual lists suggested and recommended species to be used in specified tests. The decision concerning which species are required for tests currently rests with the EPA Regions and Corps of Engineers Districts, which must consider local factors in determining species appropriateness. This is done through the development of local testing manuals.

   b. Guidance for performing tests includes discussion of: species selection, apparatus, experimental conditions, sample preparation, test design, QA/QC, and data presentation and interpretation.

   c. Water column tests

      i. Elutriate chemical concentrations are used to assess compliance with water quality criteria; the water quality criteria are considered the "Limiting Permissible Concentration" not to be exceeded after consideration of initial mixing.

      ii. Elutriate bioassays are static 96 hour LC50 studies using three concentrations of elutriate; 1% of the LC50 concentration is considered the "Limiting Concentration" not to be exceeded after consideration of initial mixing.

      iii. The regulations require at least one species from phytoplankton or zooplankton, crustacean or mollusc, and fish. A range of test species are suggested, and a subset is recommended, in the testing manual for water column bioassays. However, decisions concerning the choice of test organisms currently rest with local decision makers.

d. Whole Sediment Bioassays

i. Whole sediment bioassays, both acute 10 day bioassays and 10 or 28 day bioaccumulation, are described in the testing manual.

ii. For both acute 10 day bioassays and 10 or 28 day bioaccumulation, the dredged material results are compared to reference site sediment results. This is done to ensure that no unreasonable degradation will occur because of disposal.

iii. The regulations require that test species comprise filter-feeding, deposit-feeding, and burrowing organisms. A range of test species are suggested, and a subset is recommended, in the testing manual for whole sediment bioassays. However, the decision concerning choice of test organisms currently rests with local decision makers.

- For acute toxicity bioassays, infaunal amphipods, burrowing polychaetes, molluscs, and crustaceans are suggested.

- For bioaccumulation tests, polychaetes, molluscs, and crustaceans are suggested.

2. 301(h) monitoring program guidance documents include a number of volumes that may be relevant to contaminated sediment testing:

a. 301(h) toxic effects of sewage discharge on coral reef communities.

b. Summary of U.S. EPA approved methods to demonstrate compliance with applicable water quality standards.

c. QA/QC for 301(h) monitoring program. Guidance on field and lab methods.

d. Bioaccumulation series (5 volumes). Addresses target species, detection limits, analytical methods, and sample replication.

II. Wetlands Division

A. Regulatory Responsibilities:

1. Develop guidelines to evaluate proposed discharges of dredged or fill material into waters of the United States.

B. Statutory and Regulatory Authority:

1. Clean Water Act, Section 404

2. Section 404(b) (1) guidelines (40 CFR 230)

C. Major Needs and Requirements for Standard Sediment Testing Methods:

1. Interest lies in testing dredged material to determine possible effects of discharge.

2. The program would like a tiered testing approach to evaluation of dredged material proposed for discharge. Needs and requirements are similar to the ocean disposal program. However, freshwater and estuarine species should also be selected for test development.

D. Existing Program Guidance and Tests Used to Assess Sediment Contamination:

1. Currently there is no broadly applied testing manual for dredged material evaluation. Evaluations are handled on a case-by-case basis using 404 (b) (1) Guidelines. However, EPA and the Corps of Engineers are in the process of developing the Inland Testing Manual to evaluate proposed discharges of dredged material into waters inside the baseline of the Territorial Sea. The manual will be completed in 1993. It incorporates a tiered testing strategy.

2. The 404 (b) (1) Guidelines provide a general framework under which testing is to be performed. The general framework includes: evaluation of existing information, chemical and biological testing (chemical characterization of material, elutriate testing, and benthic effects testing), and evaluation of physical effects of disposal.

3. Regional guidance for testing dredged material has been developed by: Regions 1, 5, 9, and 10.

   a. Region 1 protocols have been developed for: selection of sampling sites; physical and bulk chemical analysis of sediments; tiered evaluation testing for liquid phase assay, suspended particulate assay, whole sediment assay, and bioaccumulation analysis; elutriate testing procedures; and QA/QC measures.

   b. Region 9 has developed guidance similar to Region 1 guidance.

c. Region 5 has produced guidance for sampling and testing efforts related to navigational dredging, and also uses the IJC Guidelines and Register for Evaluation of Great Lakes Dredging Projects.

d. Region 10 has developed the Puget Sound Dredged Disposal Analysis, which includes a tiered battery of tests.

e. Other Regions apply variations of the ocean disposal testing manual to dredged material assessment programs in waters of the United States.

4. It should be noted that standardized testing in the ocean dumping, 404, and other programs must address the interfering effects of sediment grain size, ammonia, and hydrogen sulfide. These are important issues for standardization.

III. Office of Pollution Prevention and Toxic Substances

A. Regulatory Responsibilities:

1. Assessment of risks resulting from possible releases of existing and new chemicals that are manufactured, distributed, or disposed.

2. Decisions to regulate the use of new and existing chemicals.

B. Statutory Authority:

1. Toxic Substances Control Act, Section 4, 5, 6, 8

C. Major Needs and Requirements for Standard Sediment Testing Methods:

1. OPPT is interested in fate, transport and effects of potential sediment contaminants. Spiked sediment testing is the kind of standardized test that would be of the greatest use in these evaluations.

2. The PCB program is also interested in developing toxicity assays for use with sediments taken from sites contaminated with PCBs. If a disposal method such as bioremediation is used to remove PCBs, toxicity tests are necessary to ensure that PCBs have been destroyed, and that intermediates more toxic than the original PCBs have not been formed.

3. Since spiked sediment tests would be of greatest use to the program, standardization of the test protocol elements described below would be most useful.

4. Sediment testing for one species is described in the program guidelines. Standardization of methods for additional species would offer a greater range of testing options.

D. Existing Program Guidance and Tests Used to Assess Sediment Contamination:

1. TSCA Guidelines used to develop data on the toxicity and bioavailability of chemical substances and mixtures.

2. Section 795.135 of the Guidelines describes the chironomid Sediment Toxicity Test.

3. Chironomid sediment test guideline describes three tests:

   a. 14-day chironomid aqueous exposure test with minimal sediments, foods, and test substance added to the water.

   b. 14-day exposure with test substance added to the sediment.

   c. 14-day interstitial exposure with the test substance added to the water.

4. The guidelines include the following key protocol elements:

   a. Conducting range finding tests;

   b. Conducting definitive tests (number of test organisms, specification for controls, test duration, endpoints, water quality measurements);

   c. Measurement of test substance;

   d. Test conditions and selection of organisms

      i. Acquisition, feeding, loading, care and handling, acclimation, facilities;

   e. Test substance delivery system;

   f. Dilution water;

g. Cleaning test system;

h. Sediments used for test, determination of contaminant partitioning and bioconcentration;

i. Additional test parameters and measurements;

j. Reporting test results.

IV. Office of Pesticide Programs

A. Regulatory Responsibilities:

1. Review the uses of new and existing chemicals to be registered as pesticides in order to determine effects on nontarget organisms.

2. Make decisions to: 1) label pesticides in order to control or restrict their use; 2) prohibit registration of new chemicals or uses; and 3) cancel registrations or ban the use of existing pesticides.

B. Statutory Authority:

1. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

C. Major Needs and Requirements for Standard Sediment Testing Methods:

1. The pesticide program is primarily interested in spiked sediment toxicity tests and dose response relationships to determine how varying application rates and uses of chemicals would affect exposed species. Standardization of spiking methods would therefore be very useful.

2. The program requires standardized tests to discriminate among exposure pathways in order to determine the bioavailability of contaminants resulting from different levels and methods of pesticide application.

3. OPP is also interested in evaluating contaminated sediment in the field in order to trace runoff of pesticides applied in agricultural practices.

4. Currently, no standardized sediment toxicity tests have been developed by the program for evaluation of pesticides.

5. The program would like to see standardization of marine and freshwater tests for both the acute and chronic studies. Representative species of appropriate sensitivity from a range of sites would have to be available for testing. Acute water column testing currently requires an invertebrate, and a warm water and cold water fish species. In addition, oyster embryolarvae, oyster shell deposition, fish early life stage, and fish full life cycle tests are currently used for water column testing. The range of sediment tests required would probably be similar to those mentioned above.

6. Mesocosm tests have also been developed by OPP for dose-response studies of pesticides.

7. A major concern of the program is that standardized sediment studies would have to be legally defensible if Agency decisions were challenged by the agricultural or chemical industries.

8. A major issue for the program in sediment testing is the kind of sediment to use in spiked testing, how the sediment should be handled, and how the contaminant should be introduced to the test system.

9. The pesticide testing guidelines and standard evaluation procedures developed by OPP provide the level of detail that would be required in standardization. This kind of guidance is generally of the same level of detail as an ASTM standard method.

10. The level of detail in a standardized test that is likely to be most useful to the pesticide program would be that of the ASTM standard practice. A standard guide may not offer enough structure, and a standard method may not be flexible enough.

D. Existing Program Guidance and Tests Used to Assess Sediment Contamination:

1. The pesticide program currently has no existing guidance for sediment testing. No standard tests or species have been developed. The program has authority to ask for such a study under special test requirements, but generally has not required sediment bioassays. If they are required, the pesticide registrant is asked to submit a protocol for evaluation by the program.

2. The Office of Pesticide Programs Standard Evaluation Procedure for Ecological Risk Assessment describes how the results of contaminated sediment studies would be used to complete a risk assessment.

V. Office of Emergency and Remedial Response (Superfund)

A. Regulatory Responsibilities:

1. Cleanup of hazardous waste sites to protect human health, welfare, and the environment.

2. Sediment assessment methods are identified and applied both for site assessments and remedy selection.

3. Once contaminants are identified, existing state and federal standards are evaluated for applicability to the site. When standards are not available, other evaluation methods are used to determine if the environment is endangered and to select cleanup goals.

B. Statutory Authority:

1. Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), as amended by the Superfund Amendments and Reauthorization Act (SARA).

2. CERCLA mandates that sampling be conducted to characterize the release of hazardous substances from a site and to determine if these releases present a threat to human health, welfare, and the environment.

C. Major Needs and Requirements for Standard Sediment Testing Methods:

1. The program is looking for a battery of test methods that have been peer reviewed and validated. These would be used with standard chemical analytical methods. A list of standardized biological methods that could be included in the Superfund Contract Laboratory Program would be very useful.

2. The Superfund Program handles a wide variety of sites, requiring a wide spectrum of testing options to meet site specific goals. More emphasis and resources are being placed on ecological assessment, and the program will be doing more quantitative assessment. Any standard methods that can be of use in these assessments will be important to the program.

3. The following standardized tests would be useful for the program:

a. Chemical Testing

i. Sediment analysis to determine bioavailability of contaminants.

ii. Total Organic Carbon (TOC).

iii. Residue Analysis

b. Biological Testing

i. Bioaccumulation Tests

ii. Solid Phase Toxicity Tests

D. Existing Program Guidance and Tests Used to Assess Sediment Contamination:

1. Superfund RI/FS Guidance and the Environmental Evaluation Manual provide general guidance on methodologies used to determine the nature and extent of sediment contamination at sites. Ecological Assessment of Hazardous Waste Sites, EPA/600/3-89/013.

2. The Data Quality Objectives Guidance and Compendium outline a detailed description of the investigation process. A Quality Action Plan (QAP) is developed on each site, and may be carried out by the EPA established contract laboratory program (CLP) or a non CLP laboratory that meets the data quality objectives of the investigation.

3. Currently, the Superfund Program draws upon several sources for testing methods. These include, but are not limited to ASTM, OECD, the U.S. Army Corps of Engineers, EPA Methods for Measuring Acute and Chronic Toxicity of Effluent Waters, and the Canadian ministry of the Environment. A list of those available for sediment tests is attached. The ASTM sediment methods are standard guides.

4. Region 4 Standard Operating Procedures for Toxicity Testing/Hazardous Waste Assessments. April 15, 1990. Written by Todd Harris, Jay Glover, Jim Maudsley, with foreword by Bill Peltier. U.S. EPA Region 4 Environmental Services Division and NSI Technology Services Corp.

VI. Office of Solid Waste

A. Regulatory Responsibilities:

1. Under the Resource Conservation and Recovery Act (RCRA), contaminated sediments exhibiting RCRA toxicity must be managed in a RCRA permitted or interim status facility.

2. Authority to enforce cleanup of contaminated sediments is used under two conditions: 1) if the sediment is dredged and exhibits a hazardous waste characteristic under RCRA, or if the sediment is mixed with a RCRA listed hazardous waste; and 2) if the sediment contamination can be shown to have resulted from a release form a specified solid waste management unit at a RCRA permitted or interim status hazardous waste facility.

3. Contaminated sediments may be toxic under the toxicity characteristic leaching procedure test, a test that compares the concentrations of various chemicals in the leachate from the dredged materials with levels established to protect the environment.

B. Statutory Authority:

1. Resource Conservation and Recovery Act (RCRA), Sections 3004 (u) and (v), and Section 7003.

C. Major Needs and Requirements for Standard Sediment Testing Methods:

1. If new sediment quality assessment methods receive adequate scientific review, OSW would incorporate them in making RCRA permit decisions, and in evaluation of remedial site restoration plans.

2. The program would be interested in tests that can link toxicity to a source of contamination. Toxicity identification evaluation would be useful.

D. Existing Program Guidance and Tests Used to Assess Sediment Contamination:

1. The program relies on the Superfund guidance described above. No standard program approaches to evaluating or testing sediment contamination have been developed. Testing decisions are made on a case-by-case basis. The program would be interested in a range of testing options that could provide the appropriate choice for a particular site.

VII. Office of Wastewater Enforcement and Compliance

A. Regulatory Responsibilities:

1. NPDES permits issued under the Clean Water Act can be written to protect against sediment contamination. Sediment testing and monitoring can be required as a condition of a discharge permit.

2. At present, water quality based effluent limits protect against sediment contamination only to the extent that such contamination would cause violations of water quality criteria.

B. Statutory Authority:

1. Clean Water Act, Sections 101(a) (3), 301 (b) (1) (C), 402, and 304 (1).

C. Major Needs and Requirements for Standard Sediment Testing Methods:

1. Acute and chronic sediment bioassays and bioaccumulation studies could be used in the NPDES permitting process.

D. Existing Program Guidance and Tests Used to Assess Sediment Contamination:

1. Sediment quality criteria are being developed to be used in NPDES permitting.

2. The guidance document, "Assessment and Control of Bioconcentratable Contaminants in Surface Waters," March 1991, describes methods for sampling and measuring bioconcentratable chemicals in sediment and biota at point source discharge sites.

PROGRAM OFFICES RESPONSIBLE FOR SEDIMENT TOXICITY TESTING

- OFFICE OF WETLANDS, OCEANS, AND WATERSHEDS
  - OCEANS AND COASTAL PROTECTION DIVISION
  - WETLANDS DIVISION
- OFFICE OF WASTEWATER ENFORCEMENT AND COMPLIANCE
- OFFICE OF POLLUTION PREVENTION AND TOXIC SUBSTANCES
- OFFICE OF PESTICIDE PROGRAMS
- OFFICE OF EMERGENCY AND REMEDIAL RESPONSE
- OFFICE OF SOLID WASTE

OFFICE OF WETLANDS, OCEANS , AND WATERSHEDS - OCEANS AND COASTAL PROTECTION DIVISION

STATUTORY AUTHORITY AND REGULATORY RESPONSIBILITY

- DETERMINE ACCEPTABILITY OF MATERIALS (INCLUDING DREDGED MATERIALS) FOR OCEAN DUMPING
- MARINE PROTECTION RESEARCH AND SANCTUARIES ACT (MPRSA)
- OCEAN DUMPING REGULATIONS (40 CFR 220-229)

OCEANS AND COASTAL PROTECTION DIVISION REQUIREMENTS FOR STANDARD SEDIMENT TESTING METHODS

- CHEMICAL ANALYTICAL TESTING, TOXICITY BIOASSAYS, AND BIOACCUMULATION TESTS

- ORGANISM TAXA MUST BE APPLICABLE TO WIDE RANGE OF SITES AND CONTAMINANTS

- RECOMMEND USE OF AMPHIPODS FOR ACUTE SEDIMENT TOXICITY TESTS

  -- AMPELISCA ABDITA, RHEPOXYNIUS ABRONIUS

  RECOMMEND SEVERAL TAXA FOR BIOACCUMULATION TESTS

  -- POLYCHAETES (NERIES SPECIES)

  -- MOLLUSCS (MACOMA SPECIES)

  WHOLE SEDIMENT CHRONIC BIOASSAYS NEEDED FOR OCEAN DUMPING PROGRAM

  SEDIMENT CHEMISTRY METHODS DEVELOPED FOR CWA 301(H) PROGRAM

OCEANS AND COASTAL PROTECTION DIVISION EXISTING TESTING GUIDANCE

- TESTING MANUAL, "EVALUATION OF DREDGED MATERIAL PROPOSED FOR OCEAN DISPOSAL" - "THE GREEN BOOK"
- LISTS SUGGESTED AND RECOMMENDED SPECIES FOR TESTS
- PROVIDES GUIDANCE FOR PERFORMING TESTS
  - -- SPECIES SELECTION
  - -- APPARATUS
  - -- EXPERIMENTAL CONDITIONS
  - -- SAMPLE PREPARATION
  - -- TEST DESIGN
  - -- QA/QC REQUIREMENTS
  - -- DATA PRESENTATION AND INTERPRETATION

"GREEN BOOK" TESTS

- ELUTRIATE BIOASSAYS
  - -- STATIC 96 HR LC50 STUDIES
- WHOLE SEDIMENT BIOASSAYS
  - -- 10 DAY BIOASSAYS
  - -- 10 OR 28 DAY BIOACCUMULATION TESTS
- DREDGED MATERIAL RESULTS ARE COMPARED TO REFERENCE SITE SEDIMENT TEST RESULTS

OFFICE OF WETLANDS, OCEANS, AND WATERSHEDS -- WETLANDS DIVISION

STATUTORY AUTHORITY AND REGULATORY RESPONSIBILITY

- TESTING DREDGED MATERIAL TO DETERMINE EFFECTS OF DISCHARGE
- CLEAN WATER ACT SECTION 404
- SECTION 404(B)(1) GUIDELINES (40 CFR 230)

WETLANDS DIVISION EXISTING PROGRAM GUIDANCE

- CURRENTLY NO BROADLY APPLIED TESTING MANUAL
- EPA AND COE NOW DEVELOPING THE "INLAND TESTING MANUAL" TO EVALUATE PROPOSED DISCHARGES OF DREDGED MATERIAL
- 404(B)(1) GUIDELINES PROVIDE GENERAL TESTING FRAMEWORK
- EPA REGIONS 1, 5, 9, AND 10 HAVE DEVELOPED GUIDANCE FOR DREDGED MATERIAL TESTING

OFFICE OF POLLUTION PREVENTION AND TOXIC SUBSTANCES

STATUTORY AUTHORITY AND REGULATORY RESPONSIBILITY

- ASSESSMENT OF RISKS FROM RELEASE OF EXISTING AND NEW CHEMICALS THAT ARE MANUFACTURED, DISTRIBUTED, OR DISPOSED
- TOXIC SUBSTANCES CONTROL ACT, SECTIONS 4, 5, 6, AND 8

OFFICE OF POLLUTION PREVENTION AND TOXIC SUBSTANCES REQUIREMENTS FOR STANDARD SEDIMENT TESTING METHODS

- INTERESTED IN FATE, TRANSPORT, AND EFFECTS OF SEDIMENT CONTAMINANTS
- SPIKED SEDIMENT TESTING IS OF GREATEST USE
- PCB PROGRAM INTERESTED IN TOXICITY ASSAYS FOR PCBS

OFFICE OF POLLUTION PREVENTION AND TOXIC SUBSTANCES EXISTING TESTING GUIDANCE

- SECTION 795.135 OF TSCA GUIDELINES DESCRIBE CHIRONOMID SEDIMENT TOXICITY TEST

- TEST GUIDELINES DESCRIBE THREE TESTS

  -- 14 DAY CHIRONOMID AQUEOUS EXPOSURE TEST WITH MINIMAL SEDIMENTS AND FOOD ADDED TO THE WATER

  -- 14 DAY CHIRONOMID TEST WITH TEST SUBSTANCE ADDED TO SEDIMENT

  -- 14 DAY INTERSTITIAL EXPOSURE WITH TEST SUBSTANCE ADDED TO THE WATER

- GUIDELINES INCLUDE A NUMBER OF KEY PROTOCOL ELEMENTS

OFFICE OF PESTICIDE PROGRAMS

STATUTORY AUTHORITY AND REGULATORY RESPONSIBILITY

- REVIEW USE OF NEW AND EXISTING CHEMICALS REGISTERED AS PESTICIDES TO DETERMINE EFFECTS ON NONTARGET ORGANISMS
- MAKE DECISIONS TO LABEL PESTICIDES TO RESTRICT USE, PROHIBIT REGISTRATION OR USES, OR CANCEL/BAN USE OF EXISTING PESTICIDES
- FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT (FIFRA)

OFFICE OF PESTICIDE PROGRAMS REQUIREMENTS FOR STANDARD SEDIMENT TESTING METHODS

- PRIMARILY INTERESTED IN SPIKED SEDIMENT TOXICITY TESTS TO DEVELOP DOSE/RESPONSE RELATIONSHIPS
- REQUIRES TESTS TO DISCRIMINATE AMONG EXPOSURE PATHWAYS
- INTERESTED IN EVALUATING CONTAMINATED SEDIMENT IN THE FIELD TO TRACE PESTICIDE RUNOFF
- PROGRAM NEEDS ACUTE AND CHRONIC MARINE AND FRESHWATER TESTS
- REPRESENTATIVE SPECIES FROM A RANGE OF SITES ARE NEEDED
- MAJOR ISSUE FOR PROGRAM IS KIND OF SEDIMENT TO USE IN SPIKED TESTING AND HOW TO INTRODUCE CONTAMINANT INTO TEST SYSTEM

OFFICE OF PESTICIDE PROGRAMS EXISTING TESTING GUIDANCE

- OPP CURRENTLY HAS NO EXISTING GUIDANCE FOR SEDIMENT TESTING
- PROGRAM HAS AUTHORITY TO ASK FOR SEDIMENT STUDY UNDER THE SPECIAL TEST REQUIREMENTS
- OPP STANDARD EVALUATION PROCEDURE FOR ECOLOGICAL RISK ASSESSMENT DESCRIBES HOW RESULTS OF SEDIMENT STUDIES WOULD BE USED IN RISK ASSESSMENT

OFFICE OF EMERGENCY AND REMEDIAL RESPONSE

STATUTORY AUTHORITY AND REGULATORY RESPONSIBILITY

- CLEANUP OF HAZARDOUS WASTE SITES TO PROTECT HUMAN HEALTH, WELFARE, AND THE ENVIRONMENT
- SEDIMENT ASSESSMENT METHODS APPLIED FOR SITE ASSESSMENT AND REMEDY SELECTION
- EXISTING STATE AND FEDERAL STANDARDS USED TO SET CLEANUP GOALS, IN ABSENCE OF STANDARDS, OTHER ASSESSMENT METHODS USED
- COMPREHENSIVE ENVIRONMENTAL RESPONSE, COMPENSATION, AND LIABILITY ACT (CERCLA) AS AMENDED BY SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT (SARA)
- CERCLA MANDATES SAMPLING TO CHARACTERIZE RELEASE OF HAZARDOUS SUBSTANCES FROM A SITE

OFFICE OF EMERGENCY AND REMEDIAL RESPONSE REQUIREMENTS FOR STANDARD SEDIMENT TESTING METHODS

- PROGRAM IS LOOKING FOR BATTERY OF TEST METHODS THAT HAVE BEEN REVIEWED AND VALIDATED
- MORE EMPHASIS IN PROGRAM IS BEING PLACED ON ECOLOGICAL ASSESSMENT
- CHEMICAL TESTS NEEDED TO DETERMINE BIOAVAILABILITY OF CONTAMINANTS
- BIOACCUMULATION AND SOLID PHASE SEDIMENT TOXICITY TESTS ARE NEEDED

OFFICE OF EMERGENCY AND REMEDIAL RESPONSE EXISTING TESTING GUIDANCE

- SUPERFUND RI/FS GUIDANCE AND ENVIRONMENTAL EVALUATION MANUAL
- DATA QUALITY OBJECTIVES GUIDANCE AND COMPENDIUM
- OTHER SOURCES OF TESTING METHODS INCLUDE
  - -- ASTM, ARMY COE METHODS, EPA METHODS FOR MEASURING EFFLUENT TOXICITY, CANADIAN MINISTRY OF ENVIRONMENT
- EPA REGION 4 STANDARD OPERATING PROCEDURES FOR TOXICITY TESTING/ HAZARDOUS WASTE ASSESSMENTS

OFFICE OF SOLID WASTE

STATUTORY AUTHORITY AND REGULATORY RESPONSIBILITY

- RESOURCE CONSERVATION AND RECOVERY ACT (RCRA) SECTIONS 3004 (U) AND (V) AND SECTION 7003
- PROGRAM DETERMINES WHETHER CONTAMINATED SEDIMENT SHOULD BE MANAGED IN RCRA PERMITTED FACILITY
- PROGRAM HAS AUTHORITY TO ENFORCE CLEANUP OF SEDIMENT IF IT IS HAZARDOUS WASTE UNDER RCRA OR IF IT HAS BEEN RELEASED FROM A RCRA PERMITTED FACILITY

OFFICE OF SOLID WASTE REQUIREMENTS FOR STANDARD SEDIMENT TESTING METHODS

- PROGRAM IS INTERESTED IN TESTS LINKING TOXICITY TO A SOURCE OF CONTAMINATION
- IF NEW SEDIMENT ASSESSMENT METHODS ARE DEVELOPED THEY WOULD BE USED IN MAKING RCRA PERMIT DECISIONS, AND IN EVALUATING REMEDIAL SITE RESTORATION PLANS

OFFICE OF SOLID WASTE EXISTING TESTING GUIDANCE

- NO STANDARD PROGRAM APPROACHES TO EVALUATING SEDIMENT
- PROGRAM RELIES ON SUPERFUND GUIDANCE
- TESTING DECISIONS ARE MADE ON A CASE-BY-CASE BASIS
- A RANGE OF TESTS WOULD BE NEEDED TO PROVIDE AN APPROPRIATE TEST FOR AN INDIVIDUAL SITE

OFFICE OF WASTEWATER ENFORCEMENT AND COMPLIANCE

STATUTORY AUTHORITY AND REGULATORY RESPONSIBILTY

- ISSUES NPDES PERMITS UNDER CLEAN WATER ACT
- PERMITS CAN BE WRITTEN TO PROTECT AGAINST SEDIMENT CONTAMINATION
- SEDIMENT TESTING AND MONITORING CAN BE REQUIRED AS CONDITION OF A DISCHARGE PERMIT
- CLEAN WATER ACT SECTIONS 101(A)(3), 301(B)(1)(C), AND 304(L)

OFFICE OF WASTEWATER ENFORCEMENT AND COMPLIANCE REQUIREMENTS FOR STANDARD SEDIMENT TESTING METHODS

- ACUTE AND CHRONIC SEDIMENT BIOASSAYS AND BIOACCUMULATION STUDIES COULD BE USED IN NPDES PERMITTING PROCESS

- SEDIMENT QUALITY CRITERIA NOW BEING DEVELOPED TO BE USED IN NPDES PERMITTING

OFFICE OF WASTEWATER ENFORCEMENT AND COMPLIANCE EXISTING TESTING GUIDANCE

- "ASSESSMENT AND CONTROL OF BIOCONCENTRATABLE CONTAMINANTS IN SURFACE WATERS"
- DESCRIBES METHODS FOR SAMPLING AND MEASURING BIOCONCENTRATABLE CHEMICALS IN SEDIMENT AND BIOTA AT POINT SOURCE DISCHARGES
- SEDIMENT CRITERIA METHODOLOGY (EQUILIBRIUM PARTITIONING METHOD)
- MODELS TO BACK CALCULATE PERMIT LIMITS FROM SEDIMENT CRITERIA

EPA Regional Sediment Needs

William Peltier, U.S. EPA Region IV, Environmental Services Division - Athens, GA

I. Present Regional Activities

A. Superfund
B. MPDES
C. Dredge material
D. Special investigations

II. Standardized Test Methods

A. ASTM
B. EPA
C. COE/EPA
D. Modification of existing methods

III. Reference Sediment

A. Regional periodic reference area
B. Synthetic reference sediment

IV. Control Sediment

A. Synthetic sediment
B. Site sediment
C. Regional site sediment

V. Species Selection

A. Standard test species
B. Regional test species
C. Criteria for alternate test species

VI. Reference Toxicant Testing

A. Selection of chemicals for reference testing
B. Required series of chemicals used in reference testing for Regional or alternate test species

VII. Test Conditions

A. Summary of test conditions and test acceptability for each selected test species

VIII. QA/AC Program

A. Contract laboratory evaluations
B. Accreditation of laboratories

IX. Sample Collection, Preservation and Holding

A. Consistency in collection methods
B. Preservation and holding times of sediment

X. Bioaccumulation

A. Standardized sampling protocols
B. Minimum detection levels with available analytical methods

XI. Technical Transfer

A. Regional and State program assistance

XII. Regional Resources

A. Present Regional and State staffing
B. Facilities and future initiatives

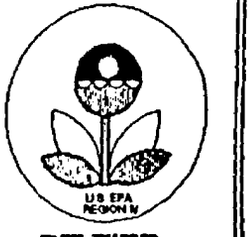
REGIONAL ACTIVITIES

SUPERFUND
NPDES RECEIVING WATER
WATER QUALITY MONITORING
NON-POINT DISCHARGES
ELUTRIATE
OCEAN DISPOSAL
INLAND AND NEAR COASTAL DISPOSAL

PELTIER

STANDARD METHODS

SELECTION - US FWS, ASTM, EPA, COE
CONSISTENCY
MODIFICATION OF EXISTING METHODS
DEVELOPMENT OF NEW METHODS
INTRA-INTERLABORATORY TESTS



PELTIER

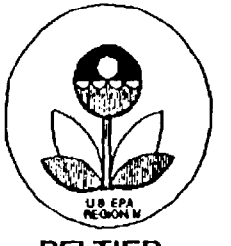
TEST CONDITIONS

SUMMARY - FEEDING, AGE, SEDIMENT DEPTH, STATIC RENEWAL ETC.

ACCEPTABILITY CRITERIA

SURVIVAL - 80% OR 90%

UNACCOUNTED FOR ORGANISMS



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REFERENCE SEDIMENT

REGIONAL PERIODIC REFERENCE SITES
LOW SURVIVAL IN REFERENCE SITES
SYNTHETIC SEDIMENT
TIGHTEN REQUIREMENTS

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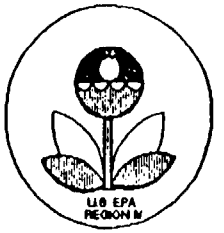
CONTROL SEDIMENT

SELECTION OF CONTROL SEDIMENT
SYNTHETIC SEDIMENT
CONTROL VS. REFERENCE



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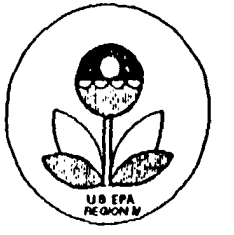
PORE WATER TESTING

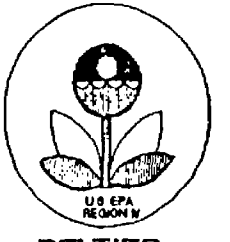
STANDARD EXTRACTION METHOD
SCREENING EVALUATION
SIGNIFICANCE TO SEDIMENT TESTING



PELTIER

SPECIES SELECTION

STANDARD TEST SPECIES
REGIONAL SPECIES
ALTERNATE TEST SPECIES CRITERIA
BRACKISH WATER SPECIES
SPECIES SENSITIVITY
REVIEW OF EXISTING TEST SPECIES



PELTIER

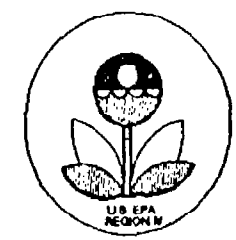
REFERENCE TOXICANT TESTING

WATER COLUMN TEST
STANDARD REFERENCE SEDIMENT
SINGLE REFERENCE TEST
FREQUENCY OF REFERENCE TESTING

PELTIER

QA/QC PROGRAM

LABORATORY EVALUATION
LABORATORY ACCREDITATION
UNKNOWN REFERENCE TOXICANT



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BIOACCUMULATION

INDIGENOUS COLLECTION

IN-SITU EXPOSURE

STANDARD METHODS FOR COLLECTION, SAMPLE QUANTITY, PROCESSING, ETC.

MINIMUM DETECTION LEVEL

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SEDIMENT SAMPLING, HOLDING, ETC

CONSISTENCY IN COLLECTION
FREEZING VS. REFRIGERATION
HOLDING TIMES

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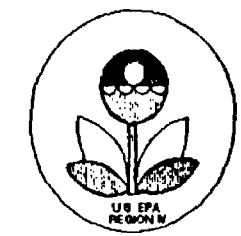
TECHNOLOGY TRANSFER

HQ POLICY, GUIDANCE, AND
REGULATIONS
REGIONAL AND STATE OUTREACH



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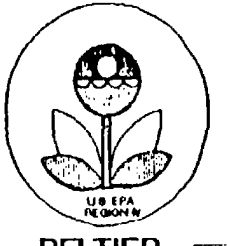
REGIONAL RESOURCES

STAFFING
CONTRACTORS
FACILITIES



PELTIER


Don't EVER
GIVE UP

Tiered Sediment Testing Conceptual Overview

Elizabeth Southerland, U.S. EPA Office of Science and Technology

The Office of Water (OW), the Office of Pesticide Programs (OPP), the Office of Pollution Prevention and Toxic Substances (OPPTS), the Office of Solid Waste (OSW), and the Office of Emergency and Remedial Response (OERR) have all committed to the principle of consistent tiered sediment testing in the Agencywide Contaminated Sediment Management Strategy. Consistent testing is desirable because all EPA programs would generate comparable data. Tiered testing would include a hierarchy of tests with the tests in each successive tier becoming progressively more rigorous, complex, and costly. Program specific interpretative guidance would be developed to explain how a decision could be made at the end of each tier on whether a sediment poses a risk that would trigger a regulatory action.

CONSISTENT AGENCYWIDE SEDIMENT TESTING

OFFICES COMMITTED TO PRINCIPLE IN AGENCYWIDE CONTAMINATED SEDIMENT STRATEGY:

- OFFICE OF WATER
- OFFICE OF PESTICIDE PROGRAMS
- OFFICE OF POLLUTION PREVENTION AND TOXIC SUBSTANCES
- OFFICE OF SOLID WASTE
- OFFICE OF EMERGENCY AND REMEDIAL RESPONSE

CONSISTENT AGENCYWIDE SEDIMENT TESTING

- EPA PROGRAMS AGREE ON WHETHER A SEDIMENT POSES AN ECOLOGICAL OR HUMAN HEALTH RISK
- COMPARABLE DATA GENERATED
- UNIFORM BASIS FOR AGENCY DECISIONS BUT EACH PROGRAM DECIDES WHETHER RISK TRIGGERS ACTION

TIERED TESTING

- HIERARCHY OF TESTS
- TIERS OF PROGRESSIVELY MORE RIGOROUS AND COMPLEX TESTS
- TIERS OF PROGRESSIVELY MORE COSTLY TESTS

INTERPRETIVE GUIDANCE FOR TIERED TESTING

- EXPLAINS HOW A DECISION IS MADE AT END OF EACH TIER ON WHETHER A SEDIMENT POSES RISK THAT TRIGGERS ACTION
- PROGRAM -SPECIFIC GUIDANCE
  - -- WEIGHT OF EVIDENCE
  - -- PASS/FAIL
  - -- REFERENCE COMPARISONS

EPA OW/COE
DREDGED MATERIAL TESTING

TIER 1 - REVIEW OF EXISTING CHEMICAL AND BIOLOGICAL DATA AND/OR INVENTORY OF NEARBY SOURCES

TIER 2 - CHEMICAL DATA GENERATED

- WATER QUALITY AND SEDIMENT QUALITY CRITERIA COMPARISONS

TIER 3 - ACUTE TOXICITY AND BIOACCUMULATION BIOASSAY DATA GENERATED

- REFERENCE AREA COMPARISONS

TIER 4 - SITE-SPECIFIC FIELD STUDIES

EPA OFFICE OF PESTICIDES AQUATIC RISK TIERED TESTING

TIER 1 - ACUTE TOXICITY DATA GENERATED

TIER 2 - CHRONIC TOXICITY (EARLY LIFE STAGE) DATA GENERATED

TIER 3 - CHRONIC TOXICITY (FULL LIFE CYCLE) DATA GENERATED

TIER 4 - FIELD OR MESOCOSM TESTING

COMPONENTS OF SEDIMENT TIERED TESTING

- ACUTE AND CHRONIC TOXICITY BIOASSAYS
- BIOACCUMULATION BIOASSAYS
- CHEMICAL CRITERIA
- OTHERS ?
  - BENTHIC COMMUNITY STRUCTURE
  - COLONIZATION RATE
  - IN SITU SEDIMENT TESTING WITHIN A MESOCOSM

Summary of ASTM Activities on Freshwater and Marine Sediment Test Methods

Chris Ingersoll, U.S. Fish and Wildlife Service, NFCR - Columbia, MO

I. Objectives

A. Overview ASTM subcommittee E47.03 on sediment toxicology
B. Standardization of sediment testing

II. Advantages of Standardization

A. Use of uniform testing procedures (e.g., dilution water, duration)
B. Increase data accuracy and precision
C. Facilitate test replication
D. Increase comparative value of results
E. Greater regulatory and legal impact

III. Disadvantages of Standardization

A. Sediment testing in infancy relative to aquatic testing
B. Inhibit creative approaches
C. Inadequate characterization of effects because of optimized conditions

IV. American Society for Testing and Materials

A. Goal: "Develop standards on characteristics and performance of products, systems, and services; promotion of related knowledge ... voluntary consensus." Publication: Annual Book of ASTM Standards, Volume 11.04

V. ASTM Definitions

A. Standard: Document development within principals of the society = consensus
B. Guide: Series of options, no recommended course of action
C. Test Method: Definitive procedure for measuring characteristics

VI. Balloting a Document by Voting Members

A. Consensus at 4 levels
   1. Task group (1 to 10 individuals
   2. Subcommittee (100 individuals + 500)
   3. Main committee (250 individuals)
   4. Society (33,000 individuals)

B. Re-ballot every 3 years (at minimum)

VII. ASTM E47.03 Sediment Toxicology Subcommittee

A. Inception: May 1987

B. Goal: "Develop guides for assessing the bioavailability of contaminants associated with sediments ... evaluate hazard of contaminated sediment, soil, sludge, drilling fluids, and similar materials."

C. Meeting schedule: Spring -- during annual ASTM symposium
   Fall -- weekend before SETAC

VIII. Approved ASTM Standards

A. E 1367-92: Standard Guide ... 10-d Toxicity with Marine & Estuarine Amphipods

B. E 1383-92: Standard Guide ... Toxicity with Freshwater Invertebrates

C. E 1391-90: Standard Guide ... Collection, Storage, Characterization, Manipulation

IX. Documents in Balloting Process (Task Groups)

A. Designing Biological Tests (Dwyer; Main/Subcommittee)
B. Toxicity Tests with Polychaetes (Reish; Main/Subcommittee)
C. Toxicity Tests with Mayflies (Bedard; Main/Subcommittee)
D. Terminology (Ingersoll; Main/Subcommittee)
E. Bioaccumulation by Benthic Invertebrates (Lee; Subcommittee)
F. Bioaccumulation by Fish (Mac; Subcommittee)

X. Documents Proposed

A. Toxicity Tests with Oysters (Dinnel)
B. Toxicity Tests with Echinoderms (Dinnel)
C. Toxicity Tests with Earthworms (Callahan)
D. Toxicity Tests with Microtox (Evereklian)
E. Toxicity Tests with Tubifex tubifex (Day)
F. Sediment Resuspension (Burton)
G. Statistical Guidance (Schlekat)
H. Toxicity Identification and Evaluation (??)

XI. E 1367: Marine and Estuarine Amphipods

A. Scope, significance, and use
B. Interference
C. Hazards
D. Test water, test and control sediments, test organisms
E. Experimental design, procedure, analyses
F. Acceptability and interpretation
G. Species-specific annexes

XII. E 1367: Marine and Estuarine Amphipods

A. Species-specific annexes
   - Annex 1. Rhepoxynius abronius
   - Annex 2. Eohaustorius spp.
   - Annex 3. Ampelisca abdita
   - Annex 4. Grandidierella japonica
   - Annex 5. Leptocheirus plumulosus
   - Annex 6. Hyalella azteca (proposed)

XII. E 1367: Marine and Estuarine Amphipods

A. Procedures:
   1. 10-d whole sediment, static, field contaminated/spiked sediment
   2. Endpoints = survival, reburial
B. Status: approved standard
C. Future plans:
   1. Chronic endpoints (growth, reproduction)
   2. Rhepoxynius abronius test method?

XIII. E 1383: Freshwater Invertebrates

A. Species-specific annexes
   - Annex 1. Hyalella azteca
   - Annex 2. Chironomus tentans
   - Annex 3. Chironomus riparius
   - Annex 4. Daphina sp. and Ceriodaphnia sp.
   - Annex 5. Hexagenia sp. (Main/Subcommittee)
   - Annex 6. Diporeia sp. (proposed; formerly Pontoporeia hoyi)
   - Annex 7. Lumbriculus sp. (proposed)
   - Annex 8. Oligochaeta (proposed, Tubifex tubifex)
   - Annex 9. Mollusks (proposed)

XV. E 1383: Freshwater Invertebrates

A. Procedures:
   1. Partial life cycle, static/flow-through, field contaminated/spiked sediment
   2. Endpoints = survival, growth, reproduction
B. Status: approved standard
C. Future plans:
   1. Additional species-specific annexes
   2. Hyalella azteca test method?

XVI. E1391: Collection, Storage, Characterization, Manipulation

A. Procedures:
   1. Sediment collection, transport, storage, characterization, spiking, and dilution
   2. Recommendations and limitations
B. Status: approved standard
C. Future plans: test methods?

XVII. Designing Biological Tests with Sediment

A. Procedures:
   1. Test type (e.g., whole sediment, pore water, elutriate)
   2. Sample collection, handling, and manipulation
   3. Test organisms and endpoints
   4. Experimental design
   5. Statistics, data interpretation, QA/QC
B. Status: Main/subcommittee ballot

XVIII Sediment Toxicity Tests with Polychaetes

A. Procedures:
   1. 4 to 20-d test, juvenile/adult, field contaminated/spiked sediment
   2. Endpoints = survival, growth
B. Status: Main/subcommittee ballot

XIX. Bioaccumulation By Benthic Invertebrates and Bioaccumulation By Fish

A. Procedures:
   1. 10 28-d, static/flow-through, field contaminated/spiked sediment
   2. Bioaccumulation potential vs. steady state
B. Status: Subcommittee ballots

XX. Research Needs for Standard Development

A. Multi-species comparisons
B. Inter-laboratory comparisons
C. Abiotic factors
D. Life history and chronic indicators of toxicity
E. Spiking methods and positive controls
F. Dilution studies and mixtures
G. Laboratory to in situ comparisons

OBJECTIVES

- Overview ASTM Subcommittee E47.03 on Sediment Toxicology
- Standardization of Sediment Testing

ADVANTAGES OF STANDARDIZATION

- Use of uniform testing procedures (e.g., dilution water, duration)
- Increase data accuracy and precision
- Facilitate test replication
- Increase comparative value of results
- Greater regulatory and legal impact

DISADVANTAGES OF STANDARDIZATION

- Sediment testing in infancy relative to aquatic testing
- Inhibit creative approaches
- Inadequate characterization of effects because of optimized conditions

AMERICAN SOCIETY FOR TESTING AND MATERIALS

- **GOAL:** "Develop standards on characteristics and performance of products, systems, and services; promotion of related knowledge...voluntary consensus."
- **PUBLICATION:** Annual Book of ASTM Standards, Volume 11.04

ASTM DEFINITIONS

- **STANDARD:** DOCUMENT DEVELOPED WITHIN PRINCIPALS OF THE SOCIETY = CONSENSUS
- **GUIDE:** SERIES OF OPTIONS, NO RECOMMENDED COURSE OF ACTION
- **TEST METHOD:** DEFINITIVE PROCEDURE FOR MEASURING CHARACTERISTICS

BALLOTING A DOCUMENT BY VOTING MEMBERS

- CONSENSUS AT 4 LEVELS
    1. Task Group (1 to 10 individuals)
    2. Subcommittee (100 individuals +500)
    3. Main Committee (250 individuals)
    4. Society (33,000 individuals)
- RE-BALLOT EVERY 3 YEARS (at minimum)

ASTM E47.03 SEDIMENT TOXICOLOGY SUBCOMMITTEE

- INCEPTION: May 1987
- GOAL: "Develop guides for assessing the bioavailability of contaminants associated with sediments... evaluate hazard of contaminated sediment, soil, sludge, drilling fluids, and similar materials."
- MEETING SCHEDULE:

  Spring--During Annual ASTM Symposium

  Fall--Weekend before SETAC

APPROVED ASTM STANDARDS

- E 1367-92: Standard Guide ... 10-d Toxicity with Marine & Estuarine Amphipods
- E 1383-92: Standard Guide ... Toxicity with Freshwater Invertebrates
- E 1391-90: Standard Guide ... Collection, Storage, Characterization, Manipulation

DOCUMENTS IN BALLOTING PROCESS (Task Groups)

- Designing Biological Tests (Dwyer; Main/Subcommittee)
- Toxicity Tests with Polychaetes (Reish; Main/Subcommittee)
- Toxicity Tests with Mayflies (Bedard; Main/Subcommittee)
- Terminology (Ingersoll; Main/Subcommittee)
- Bioaccumulation by Benthic Invertebrates (Lee; Subcommittee)
- Bioaccumulation by Fish (Mac; Subcommittee)

DOCUMENTS PROPOSED

- Toxicity Tests with Oysters (Dinnel)
- Toxicity Tests with Echinoderms (Dinnel)
- Toxicity Tests with earthworms (Callahan)
- Toxicity Tests with Microtox (Evereklian)
- Toxicity Tests with Tubifex tubifex (Day)
- Sediment Resuspension (Burton)
- Statistical Guidance (Schlekat)
- Toxicity Identification and Evaluation (??)

E 1367: MARINE AND ESTUARINE AMPHIPODS

A. Scope, Significance, and Use

B. Interferences

C. Hazards

D. Test Water, Test and Control Sediments, Test Organisms

E. Experimental Design, Procedure, Analyses

F. Acceptability and Interpretation

G. Species-specific Annexes

E 1367: MARINE AND ESTUARINE AMPHIPODS

- **SPECIES-SPECIFIC ANNEXES**

  Annex 1. Rhepoxynius abronius

  Annex 2. Eohaustorius spp.

  Annex 3. Ampelisca abdita

  Annex 4. Grandidierella japonica

  Annex 5. Leptocheirus plumulosus

  6. Hyalella azteca (proposed)

E 1367: MARINE AND ESTUARINE AMPHIPODS

- PROCEDURES:

  -10-d whole sediment, static, field contaminated/spiked sediment

  -Endpoints = survival, reburial

- STATUS: approved standard

- FUTURE PLANS:

  -Chronic endpoints (growth, reproduction)

  -Rhepoxynius abronius test method?

E 1383: FRESHWATER INVERTEBRATES

- **SPECIES-SPECIFIC ANNEXES**

  Annex 1. Hyalella azteca

  Annex 2. Chironomus tentans

  Annex 3. Chironomus riparius

  Annex 4. Daphnia sp. and Ceriodaphnia sp.

  Annex 5. Hexagenia sp. (Main/Subcommittee)

E 1383: FRESHWATER INVERTEBRATES

- SPECIES-SPECIFIC ANNEXES (cont.)

    6. Diporeia sp. (proposed; formerly Pontoporeia hoyi)

    7. Lumbriculus sp. (proposed)

    8. Oligochaeta (proposed; Tubifex tubifex)

    9. Mollusks (proposed)

E 1383: FRESHWATER INVERTEBRATES

- **PROCEDURES:**

  -Partial life cycle, static/flow-through, field contaminated/spiked sediment

  -Endpoints = survival, growth, reproduction

- **STATUS:** approved standard

- **FUTURE PLANS:**

  -Additional species-specific annexes

  -Hyalella azteca test method?

E1391: COLLECTION, STORAGE, CHARACTERIZATION, MANIPULATION

- PROCEDURES:

  -Sediment collection, transport, storage, characterization, spiking, and dilution

  -Recommendations and Limitations

- STATUS: approved standard

- FUTURE PLANS: Test methods?

DESIGNING BIOLOGICAL TESTS WITH SEDIMENT

- **PROCEDURES:**

  -Test type (e.g., whole sediment, pore water, elutriate)

  -Sample collection, handling, and manipulation

  -Test organisms and endpoints

  -Experimental design

  -Statistics, data interpretation, QA/QC

- **STATUS:** Main/Subcommittee ballot

SEDIMENT TOXICITY TESTS WITH POLYCHAETES

- **PROCEDURES:**

  -4 to 20-d test, juvenile/adult, field contaminated/spiked sediment

  -Endpoints = survival, growth

- **STATUS: Main/Subcommittee ballot**

BIOACCUMULATION BY BENTHIC INVERTEBRATES and BIOACCUMULATION BY FISH

- PROCEDURES:

  -10 to 28-d, static/flow-through, field contaminated/spiked sediment

  -Bioaccumulation potential vs. steady state

- STATUS: Subcommittee ballots

RESEARCH NEEDS FOR STANDARD DEVELOPMENT

- MULTI-SPECIES COMPARISONS
- INTER-LABORATORY COMPARISONS
- ABIOTIC FACTORS
- LIFE HISTORY AND CHRONIC INDICATORS OF TOXICITY
- SPIKING METHODS AND POSITIVE CONTROLS
- DILUTION STUDIES AND MIXTURES
- LABORATORY TO IN SITU COMPARISONS

EPA Approaches for Biological Methods Standardization: Historical Perspective and Present Guidance

Jim Lazorchak, U.S. EPA Environmental Monitoring and Systems Laboratory - Cincinnati, OH

I. **Background - Standardization of Biological Methods 1960 - 1989**
   - A. National Water Quality Network (NWQN)
   - B. Methods Development and Quality Assurance Research Lab
   - C. Environmental Monitoring and Support Laboratory - Cincinnati
   - D. Environmental Monitoring Systems Laboratory - Cincinnati
   - E. Biological Advisory Committee
   - F. Biological Methods Manual (1973)

II. **Whole Effluent Toxicity Testing Manuals**

   A. Research Method to 304(H) CWA Approved Method (1981-1992)

III. **Current Status of Biological Methods Standardization**

   A. EPA Biological Advisory Committee Charter

IV. **Existing Agency (ORD, 1987) Guidance on Methods Standardization/Validation**

   A. Six Steps:

      1. Determination of Method Requirements and DQO's
      2. Method Selection/Development
      3. Single-Laboratory Evaluation
      4. Confirmatory Testing
      5. Interim Methods Description
      6. Formal Collaborative (interlaboratory study)

V. **New EMMC Workgroup on Biological Methods Integration**

OUTLINE OF ACTION TAKEN (1989-1991)

MAY 20, 1992

1.	12/4/89	-	PUBLICATION OF PROPOSED RULE
2.	2/4/90	-	PUBLIC COMMENT PERIOD CLOSED
3.	3/90	-	PUBLIC COMMENTS SUMMARIZED
4.	4/90	-	FOUR TECHNICAL SUBGROUPS ORGANIZED (AQUA.TOX., STAT, AMES TEST, VIRUSES)
5.	1/91	-	RESPONSES TO PUBLIC COMMENTS COMPLETED
6.	5/7/91	-	FINAL RULE TO RED BORDER REVIEW (OPPE, OPTS, OGC, OW, REG. 3 & 65) (NEW LAWYER, REGAS, ASSIGNED)
7.	5/27/91	-	RED BORDER REVIEW COMPLETE (OW AND OGC NON-CONCUR)
8.	6/91	-	FURTHER ACTION ON RULE PLACED ON HOLD UNTIL TOXICITY TEST MANUALS ARE REVISED

BIOLOGICAL METHODS TO BE INCLUDED IN 304 (H)

MAY 20, 1992

PROPOSED ACTION:

1. TOXICITY TEST METHODS
   - A. ACUTE AND CHRONIC TOXICITY TESTS FOR EFFLUENTS AND RECEIVING WATERS
   - B. ACUTE TOXICITY TESTS FOR DRILLING MUDS
   - C. AMES (MUTAGENICITY) TEST FOR EFFLUENTS, RECEIVING WATERS, AND SLUDGES.

2. METHODS FOR COLLECTION, CONCENTRATION, ENUMERATION, AND IDENTIFICATION OF VIRUSES IN SURFACE WATERS, WASTEWATERS, AND SLUDGES.

3. UPDATED REFERENCES FOR BACTERIOLOGICAL TESTS

INCLUSION OF TOXICITY TESTS IN 304 (H)

MAY 20, 1992

RESULTS OF 1991 RED BORDER REVIEW:

1. OPPE & OPTS* CONCURRED WITHOUT COMMENT

2. REGIONS 3 & 5 CONCURRED WITH MINOR COMMENTS

3. OW CONCURRED CONTINGENT ON COMPLETION OF REVISED TOXICITY TEST MANUALS BEFORE THE FINAL RULE IS PUBLISHED

4. OGC NON-CONCURRED. CONCURRENCE DEPENDS ON:
   - A. REVISION/APPROVAL OF FINAL RULE
   - B. REVISION/APPROVAL OF RESPONSES TO PUBLIC COMMENTS
   - C REVISION/APPROVAL/PUBLICATION OF MANUALS

* OPTS is now the Off. Poll. Prevention & Toxics

STATUS OF INCLUSION OF TOXICITY TESTS IN 304 (H)

MAY 20, 1992

ACTION SINCE 1991 RED BORDER REVIEW:

1. 9/91 - REVISED ACUTE MANUAL SENT TO PRINTER
2. 9/91 - NEW OGC LAWYER (SWEENEY) ASSIGNED
3. 1/92 - CHRONIC MANUALS NEAR COMPLETION
4. 2/92 - ACTION ON RULE RESTARTED (MEETING IN DC WITH OGC, OWEC)
   - REVISION OF COMMENTS AND RULE
   - HARMONIZATION OF POLICY IN MANUALS, RESPONSE TO COMMENTS, AND FINAL RULE

TARGET DATES FOR COMPLETION AND PUBLICATION OF RULE

5. 5/92 - COMPLETION OF REVISED RESPONSE TO COMMENTS (RTC) AND FINAL RULE (FR)
6. 6/92 - REVIEW OF RTC AND FR BY 304H WKGP
7. 7/92 - RED BORDER REVIEW
8. 8/92 - SUBMISSION OF RULE TO ADMINISTRATOR
9. 12/92 - PUBLICATION OF RULE IN FED REG

BIOLOGICAL ADVISORY COMMITTEE

The vision of the Biological Advisory Committee is to provide technical advice to the agency on all biological methods and related ecological issues.

MISSION OF THE COMMITTEE

1. Review, comment, and assist Regions, ORD and Program Offices in standardizing and evaluating EPA biological methods and indicators to be used by Regional and State programs.

2. Ensure that states develop logically consistent and ecologically meaningful biological criteria that facilitate interstate, interregion and Environmental Monitoring Assessment Programs (EMAP) national comparisons.

3. Exchange technical information and experience in the collection, analyses, and use of biological methods and indicators in assessing the effects of impacts on biological integrity.

4. Review, comment, and assist Regions and Program offices in developing agency biological monitoring or biocriteria policy.

5. Represent agency for ecological methods and other related issues on National EPA and other Federal committees (e.g., EPA Environmental Monitoring Management Council (EMMC) and OMB Intergovernmental Task Force on Monitoring Water Quality).

Biological Advisory Committee (BAC) will be headed by a Chairperson from EMSL-Cincinnati, Deputy Chairperson from ORD or a Program Office.

Each subcommittee will have a chairperson and vice-chairperson, one of which is:

1. an ORD or Program Office Representative, and
2. a Regional Representative

The following subcommittees were agreed upon by the BAC during the FY91 meeting:

Toxicity Assessment Subcommittee:

This subcommittee's mission is to address methods and other issues related to assessing acute toxicity, chronic toxicity, bioaccumulation, and organism physiological (biomarker level) dysfunction due to contaminant in water, sediment and soil.

Ecological Assessment Subcommittee:

This subcommittee's mission will be to address methods and issues that measure ecosystem health in aquatic and terrestrial systems.

The following addition of steering committees to the BAC Committee and its subcommittees was recommended by the BAC Chairperson and Subcommittee Chairpersons during a November, 1992 Society for Environmental Toxicology and Chemistry meeting:

BAC Steering Committee:

A steering committee consisting of BAC Chairperson, Deputy Chairperson and Subcommittee Chairpersons and Vice-Chairpersons, will be responsible for the development of strategic plans for organizational and technical issues. Such plans will be approved by the full BAC Committee.

Toxicity Assessment Subcommittee Steering Committee:

The mission of the steering committee will be to organize, prioritize, and expedite subcommittee activities.

Ecological Assessment Subcommittee Steering Committee:

The mission of the steering committee will be to organize, prioritize, and expedite subcommittee activities.

ORGANIZATIONS REPRESENTED ON COMMITTEE

1. A minimum of 1-2 Biologists from each Regional Environmental Services Division (upon recommendation from each ESD and Biologist from Water Management and/or Waste Management).

2. A minimum of 1-2 Biologists from each ORD Laboratory.

3. A minimum of 1-2 Biologists from each of the following Program Offices:

    Office of Wastewater Enforcement and Permits
    Office of Science and Technology
    Office of Wetlands, Oceans and Watersheds
    Office of Solid Waste and Emergency Response
    Office of Toxic Substances
    Office of Pesticides

COMMITTEE ACTIVITIES

1. Quarterly conference calls:

    10 or less Regional Biologists plus 1-2 ORD Reps, 1-2 Hdqtrs Rep

2. Information exchange in:

    Monthly surface water monitoring status report
    Regional monthly reports circulated to regional biologists
    NETAC Newsletter
    Superfund monthly FORUM report
    EMSL - Bulletin computer board
    EMAIL
    FAX

EPA BIOLOGICAL ADVISORY COMMITTEE: ORGANIZATION STRUCTURE

I. BAC Chairperson and Deputy Chairperson

II. EPA Biological Advisory Committee Steering Committee

- A. BAC Chairperson and Deputy Chairperson
- B. Subcommittee Chairpersons and Vice-Chairpersons

III. EPA Biological Advisory Subcommittees

- A. Toxicity Assessment Subcommittee
  - 1. Chairperson, Vice-Chairperson, and Steering Committee
- B. Toxicity Assessment Subcommittee Steering Committee
- C. Ecological Assessment Subcommittee
  - 1. Chairperson, Vice-Chairperson, and Steering Committee
  - 2. Ecological Assessment Subcommittee Steering Committee

COMPOSITION OF EACH EPA BIOLOGICAL ADVISORY COMMITTEE

EPA Biological Advisory Committee Chairperson:

The Chairperson of the EPA biological Advisory Committee will be the Chief of the Bioassessment & Ecotoxicology Branch.

EPA Biological Advisory Committee Deputy Chairperson:

The Deputy Chairperson of the EPA Biological Advisory Committee will be a willing ORD, Regional or Program Office Representative appointed by the Chairperson of the EPA BAC.

EPA Biological Advisory Committee Steering Committee:

The Steering Committee will consist of the present and past BAC Chairpersons and Deputy Chairperson, Chairpersons and Vice-Chairpersons of the EPA BAC Subcommittees.

COMPOSITION OF EACH EPA BIOLOGICAL ADVISORY SUBCOMMITTEE

Toxicity Assessment Subcommittee:

The subcommittee will consist of BAC members willing to serve and a Chairperson nominated by anyone on the BAC, willing to serve, and elected by the BAC. The subcommittee will also include a Vice-Chairperson that is a willing ORD, Regional or Program Office Representative nominated and elected by the members of the BAC Subcommittee. Either the Subcommittee Chairperson or Vice-Chairperson should be from and ORD Laboratory.

Toxicity Assessment Subcommittee Steering Committee:

The Subcommittee Steering Committee will consist of a minimum of 3 BAC members (in addition to Subcommittee Chairperson and Vice-Chairperson) willing to serve and confirmed by Subcommittee Chairperson and Vice-Chairperson. Steering committee should be composed of a least 1 member from each of the following organizations: Regional Office, Headquarters Program Office, and an ORD representative.

Ecological Assessment Subcommittee:

The Subcommittee will consist of BAC members willing to serve and a Chairperson nominated by anyone on the BAC, willing to serve, and elected by the BAC. The subcommittee will also include a Vice-Chairperson that is a willing ORD, Regional or Program Office Representative nominated and elected by the Subcommittee members. Either the Subcommittee Chairperson or Vice-Chairperson should be from an ORD Laboratory.

Ecological Assessment Subcommittee Steering Committee:

A minimum of 3 BAC members (in addition to the Subcommittee Chairperson and Vice-Chairperson) willing to serve and confirmed by Subcommittee Chairperson and Vice-Chairperson. Steering committee should be composed of a least 1 member from each of the following organizations: Regional Office, Headquarters Program Office and an ORD Representative.

NOTE: Tentatively the Committee and Subcommittee officers are filled by the following individuals:

*** EPA Biological Advisory Committee (BAC)**

Chairperson, Jim Lazorchak, ORD EMSL-Cincinnati
Deputy Chairperson, Vacant

*** BAC Steering Committee:**

Bill Peltier
Teresa Norberg-King
Ron Preston
Don Klemm
Cornie Weber

*** Toxicity Assessment Subcommittee:**

Chairperson, Bill Peltier, Region 4 ESD
Vice-Chairperson, Teresa Norberg-King, ORD ERL-Duluth

*** Steering Committee:**

Joe Cummins, Region 10, ESD
Cornie Weber, ORD EMSL-Cincinnati
Margarete Heber, HDQTS OW

*** Toxicity Assessment Subcommittee Members**

Peter Nolan, Region 1
Robert Donaghy, Region 3
Chick Steiner, Region 5
Terry Hollister, Robert Vickery, P. Crocker, Region 6
Mike Tucker, Region 7
Loys Parrish, Glenn J. Rodriguez, Region 8
Joe Cummins, Region 10
Gary Chapman, ORD ERL-Newport
Doug Middaugh, ORD ERL-Gulf Breeze
Don Klemm, ORD EMSL - Newtown
Phil Lewis, ORD EMSL - Newtown

-- Members signed up as of 6/91 BAC Meeting. Others not attending 6/91 meeting can also join, contact subcommittee chairperson.

*** Ecological Assessment Committee:**

Chairperson, Ron Preston, Region 3 ESD
Co-Chairperson, Don Klemm, ORD EMSL-Cincinnati\

*** Steering Committee:**

Jim Kurtenbach, Region 2, ESD
Chris Faulkner, HDTS Wetlands, Watershed, Oceans
George Gibbons, HDTS, Science and Technology

*** Ecological Assessment Subcommittee Members**

Peter Nolan, Region 1
Jim Kurtenbach, Region 2
Jim Green, Region 3
Del Hicks, Jerry Stober, Hoke Howard, Region 4
Wayne Davis, Thomas Simon, Region 5
Evan Hornig, Region 6
Gary E. Welker, Region 7
Loys Parrish, Region 8
Peter Husby, Region 9
Gretchen Hayslip, Gerald Montgomery, Region 10
Teresa Norberg-King, ORD ERL-Duluth
Chris Faulkner, OWOW AWD
Margarete Heber, OST HESD
Don Klemm, ORD EMSL - Newtown
Brian Hill, ORD EMSL - Newtown
Frank McCormick, ORD EMSL - Newtown
Phil Lewis, ORD EMSL - Newtown

-- Members signed up as of 6/91 BAC Meeting.
Others not attending 6/91 BAC meeting can also join, contact subcommittee Chairperson.

TOXICITY ASSESSMENT SUBCOMMITTEE FUNCTIONAL STATEMENT

The Subcommittee functions within the EPA EMSL-Cincinnati Biological Advisory Committee (BAC) that provides guidance in the area of toxicity assessment. The Subcommittee is directed by a Chairperson with members representing EPA HQ Offices, ORD Laboratories and Regions who volunteer to serve on the Subcommittee.

The functions of the Subcommittee are as follows:

* Assist in the preparation, and coordination of toxicity test methods prepared for publication by the EPA for application in freshwater, marine and terrestrial ecosystems. EPA activities or programs impacted are as follows: NPDES whole effluent testing, sediment testing, toxicity reduction evaluation, dredge and fill, ocean disposal, EMAP, CERCLA, RCRA, TSCA and FIFRA.

* Assess existing toxicity test methodologies (test condition, species, endpoints, and methods of data analyses) that will impact EPA National and Regional implementation and enforcement of ecological programs.

* Provide ORD laboratories and HQ programs annually with a technical assistance needs list to support Regional toxicity assessment activities associated with the NPDES program, Water Quality Standards, and sediment criteria.

* Serve the Environmental Monitoring Council (EMMC) as a resource for review of toxicity testing methods proposed by other offices. Provides information on Subcommittee activities relating to the development and use of new toxicity testing methods.

* Coordinate with the Ecological Assessment Subcommittee of the BAC on overlapping activities, such as biomarkers development and statistical analyses.

* Integrate toxicity biomarkers from the agency's strategic planning initiative into the activities of the Toxicity Assessment Subcommittee.

* Make available Subcommittee technical expertise to State and EPA Regional offices and HQ programs for: program, project, or report reviews; judicial, administrative, or legislative hearings; and adversarial proceedings.

ECOLOGICAL ASSESSMENT SUBCOMMITTEE: FUNCTIONAL STATEMENT

The Subcommittee functions within EPA EMSL-Cincinnati Biological Advisory Committee (BAC) that provides guidance in the area of ecological assessment. The Subcommittee is facilitated by a Chairperson with members representing EPA HQ program offices, ORD Laboratories and Regions who volunteer to serve on the Subcommittee.

The functions of the Subcommittee are as follows:

* Assist in the preparation, review and coordination of ecological assessment methods prepared for publications by EPA for application in freshwater, marine and terrestrial ecosystems. EPA activities or programs impacted are as follows: water quality monitoring, environmental indicators, biological criteria, non-regionalization, EMAP, CERCLA, RCRA, TSCA and FIFRA.

* Evaluate existing ecological assessment methodologies (biosurveys, field procedures, study design and methods of data analyses) that will impact EPA National and Regional activities utilizing ecological assessments.

* Provide ORD laboratories and HQ programs annually with a technical assistance needs list to support ecological assessment activities associated with EPA and State environmental protection programs.

* Serve the Environmental Monitoring Management Council (EMMC) as a resource for review of ecological assessment activities proposed by other offices. Provides information on Subcommittee activities relating to the development and use of state-of-the-art ecological assessment methods.

* Coordinate with the Toxicity Testing Subcommittee of the BAC, on overlapping activities, such as biomarkers development and statistical analyses.

* Integrate ecological indicator goals from the agency's strategic planning initiative into the activities of the Ecological Assessment Subcommittee.

* Make available Subcommittee technical expertise to State and EPA Regional offices and HQ programs for: program, project or report reviews; judicial, administrative or legislative hearings; and adversarial proceedings.

STANDARDIZATION OF BIOLOGICAL METHODS

1. INTRODUCTION

1.1 BACKGROUND

Field and laboratory methods for monitoring the status and trends of the biological integrity of aquatic communities and the quality of surface waters and effluents have played a key role in Federal and State water pollution control programs for several decades. The current Agency biological monitoring methods development and standardization program emerged from activities of the National Water Quality (Monitoring) Network (NWQN) in Cincinnati in the late 1960's, later renamed successively the Methods Development and Quality Assurance Research Laboratory (MDQARL), the Environmental Monitoring and Support Laboratory - Cincinnati, and the Environmental Monitoring Systems Laboratory - Cincinnati. Development, evaluation, standardization and publication of biological (field) sampling and (laboratory) analysis methods, first begun by the NWQN in the late 1950's, has preceded uninterrupted to the present.

The need for a formal monitoring methods development and standardization program was recognized by the federal water pollution control program (Federal Water Quality Agency) in the late 1960's. As a result, an Agency chemical methods advisory group was created to recommend and assist NWQN in the standardization and publication of chemical and physical monitoring methods for water. The first Agency manual of chemical and physical monitoring methods was published in 1968.

An Agency biological advisory committee was created by MDQARL in 1970, consisting of representatives from the regions, research laboratories, and headquarters program offices. The members of the committee were selected to provide a cross-section of technical expertise in biological monitoring and guidance on state, regional and headquarters program office requirements for aquatic biology data. At that time, the emphasis in biological monitoring in the Agency water monitoring program was on the effects of discharges from publicly owned sewage treatment plants on the structure and function of aquatic communities.

Methods for the collection and analysis of biological samples and interpretation of biological data were selected from the peer-reviewed literature and techniques then in regular use by Agency regional and research personnel and state programs. Primary emphasis was placed on taxonomic composition and standing crop. Data on the identification and enumeration of aquatic organisms were used to establish the status and trends of biological integrity in terms of indicator organisms, the proportion of sensitive (clean water) and tolerant(polluted water) organisms, and species diversity indices. The first Agency biological monitoring methods manual was published by MDQARL in 1973 (USEPA, 1973). The Biological Advisory Committee, established in 1970, has continued to function to the present.

In the mid-1970's, primary emphasis on biological monitoring in the Agency and states began to shift from the biological integrity of ambient waters to the measurement of effluent toxicity. In response to the new Agency and state programs needs, EMSL, with the assistance of the Biological Advisory Committee, published the first methods manual for monitoring the acute toxicity of effluents and surface waters (USEPA, 1978) to aquatic organisms, now in its fourth edition (USEPA, 1991), and methods manuals for the estimation of the chronic toxicity of effluents and surface waters to freshwater and marine organisms (USEPA, 1992a, 1992b), based on methods developed by the Environmental Research Laboratories at Duluth and Narragansett, respectively.

1.2 CURRENT STATUS OF BIOLOGICAL METHODS STANDARDIZATION

During this period of chemical, physical and biological methods development (21965 -to the present), the Agency was also developing and continually strengthening its quality assurance program, which rests heavily on the availability of standardized and validated methods. The development of the Agency's policy for a water quality-based approach to discharge permitting (USEPA, 1984) and the subsequent move to place toxicity limits in discharge permits and to include effluent toxicity test in the list of "official" EPA (largely chemical and physical) monitoring methods in Table I, 40 CFR Part 136, have led to questions related to biological methods standardization, such as, "when (at what point) is a biological method considered to be standardized or validated?" or, "what process is involved in biological methods standardization and validation?" The agency currently lacks an official policy on methods standardization.

1.3 EXISTING AGENCY (ORD) GUIDANCE ON METHODS STANDARDIZATION/VALIDATION

The process of monitoring methods selection, standardization and validation is essentially similar for chemical, physical and biological methods. A consensus document or "white paper" on the subject (USEPA, 1987) was prepared by the staff of the Office of Acid Deposition, Environmental Monitoring, and Quality Assurance (it has since been renamed the Office of Monitoring, Modeling and Quality Assurance, or OMMSQA), but the document and its contents have not yet been "officially" endorsed by OMMSQA or the Agency, or promulgated as Agency policy.

Because of the detail and clarity of the 1987 document, it would be senseless to repeat its contents in full, here. However, it would be advantageous to provide a summary of the process described in the document, and to indicate how the steps may differ, if at all, for biological methods.

The six major steps are described below. In the case of biological methods development/validation, the Biological Advisory Committee should be consulted during each step.

1. **Determination of method requirements and data quality objective**

   - Provided by the program office

2. **Method selection/development**

   - Potential user, such as program office and/or regions, should be actively involved.

3. **Single-laboratory evaluation**

   - Includes sensitivity to test method variables (ruggedness) and single laboratory/single operator precision.

4. **Confirmatory testing**

   - Evaluation by several (minimum of three) independent labs.

5. **Interim method description**

   - Full method description, information on ruggedness, mandatory and optional test conditions, guidance on data analysis, single laboratory precision, etc. If endorsed by the Agency, it would now be considered a "standard" method.

6. **Formal collaborative (interlaboratory study)**

   - Complete, acceptable, data from a minimum of six labs.

METHODS STANDARDIZATION

BIBLIOGRAPHY:

1. AOAC. 1984. Report of the Committee of Collaborative Interlaboratory Studies, Association of Official Analytical Chemists. J. Assoc. Anal. Chem. 67 (2)

2. ASTM. 1979. Standard practice for conducting an interlaboratory test program to determine the precision of test methods. Annual Book of Standards, 14.02. Water, Standard E691-79. American Society for Testing and Materials, Philadelphia, Pennsylvania.

3. ASTM. 1981. Standard practice of precision and accuracy of methods of Committee D-22 on Sampling and Analysis of Atmospheres. Annual Book of Standards, 11.03. Standard E3670-81. American Society for Testing and Materials, Philadelphia, Pennsylvania.

4. ASTM. 1985. Standard practice of determination of precision and accuracy of methods of Committee D-19 on Water. Annual Book of Standards, 11.01. Standard D2777-85. American Society for Testing and Materials, Philadelphia, Pennsylvania.

5. Battelle Columbus Laboratories. 1982. Development of appropriate statistical techniques to compare analytical methods across wastewaters. Bishop, T.F., F.E. Brydon, and E.C. Dutter. Battelle Columbus Laboratories, Columbus, Ohio. USEPA Contract 68-03-2624.

6. Battelle Columbus Laboratories. 1985. Single laboratory validation protocol. Battelle Columbus Laboratories, Columbus Ohio. USEPA Contract 68-03-3224, Work assignment #1.

7. Glaser, J.A., D.A. Foerst, G.D. McKee, S.A. Quave, and W.L. Budde. 1981. Trace analyses for wastewater. Environ. Sci. Techn. 15(12):1426-1435.

8. USEPA. 1983. Guidelines and format for EMSL-Cincinnati methods. J.F. Kopp. U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/4-83/020.

9. USEPA. 1983. Guidelines for conducting single laboratory evaluations of biological methods. McKenzie, W., and T. Olsson, III, Bioassay Systems Corporation. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada. EPA/600/4-83/056.

10. USEPA. 1984. The development of data quality objectives. Quality Assurance Management Staff, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. Unpublished report.

11. USEPA. 1984. Formal collaborative study design for water and wastes. P.W. Britton, ed. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA/600/4-83/020.

12. USEPA. 1987. Guidelines for selection and validation of USEPA'S measurement methods. Office of Acid Deposition, Environmental Monitoring, and Quality Assurance, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. Unpublished report. 57 pp.

13. USEPA. 1983. Validation of testing/measurement methods. L.R. Williams. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada. EPA/600/X-83/060.

STEPS IN BIOLOGICAL METHODS DEVELOPMENT, STANDARDIZATION, AND VALIDATION

1. Determination of Methods Requirements and DQO's

2. Candidate Method Selection/Development

3. Method Evaluation

    - Single Laboratory
    - Precision, Bias, Ruggedness

4. Confirmatory Testing

    - Evaluation by 3 Labs

5. Interim Method Description

    - Now a Standard Method

6. Formal Collaborative Study

    - Data from 6 Labs

BIOLOGICAL METHODS STANDARDIZATION
OUTLINE OF TALK

FWQA METHODS PROCESS 1960 - 1970

EPA METHODS PROCESS 1970 - 1989

CWA 304 (H) EPA APPROVED METHODS PROCESS

1990s EPA BIOLOGICAL ADVISORY COMMITTEE

1987 ORD GUIDANCE ON METHODS STANDARDIZATION AND VALIDATION

EMMC BIOLOGICAL METHODS INTEGRATION WORKGROUP

HISTORICAL METHODS STANDARDIZATION

Cornelius I. Weber

LATE 1950s - MONITORING METHODS - USPHS

NATIONAL WATER QUALITY (MONITORING) NETWORK - NWQN

Development, evaluation, standardization, and publication of biological field sampling and laboratory analysis methods

LATE 1960s - MONITORING METHODS - FWQA

METHODS DEVELOPMENT AND QUALITY ASSURANCE LAB

More formal process:

FWQA - Chemical Methods Advisory Group

MISSION - Recommend & assist standardization & publication - chemical/physical monitoring methods

FIRST METHODS MANUAL - CHEMICAL/PHYSICAL MONITORING METHODS - 1968

ENVIRONMENTAL MONITORING & SUPPORT LAB - CIN

ENVIRONMENTAL MONITORING SYSTEMS LAB - CIN

HISTORICAL EPA METHODS STANDARDIZATION

Cornelius I. Weber

1970 - EPA

METHODS DEVELOPMENT & QUALITY ASSURANCE RESEARCH LABORATORY - CINCINNATI

1970 Biological Advisory Committee - MDQARL

REPRESENTATION:

- EPA Regions
- EPA Research Labs
- EPA Program Offices

EXPERTISE: CROSS-SECTION OF TECHNICAL EXPERTISE

- Biological Monitoring
- Guidance on State, Regional, & Program Office requirements for aquatic biology data

MISSION: Provide technical input on methods for biological monitoring in EPA water monitoring program with emphasis on the effects of sewage treatment plant discharges on the structure and function of aquatic communities.

HISTORICAL EPA METHODS STANDARDIZATION

Cornelius I. Weber

1970s CONTINUED

1973 - MDQARL-Cincinnati Cornelius Weber publishes first biological methods manual:

"Biological field and laboratory methods for measuring the quality of surface waters and effluents."

CONTENTS:

BIOMETRICS - Statistical treatment of biological data

PLANKTON - Collection, enumeration, indices

PERIPHYTON - Collection, enumeration, indices

MACROPHYTON - Collect, count, interpret

MACROINVERTEBRATE - Collection, enumeration, indices
i.e. Pollution tolerance, diversity

FISH - Collection, count, data analyses

BIOASSAYS - Fish, invertebrates, algae, periphyton

HISTORICAL EPA METHODS STANDARDIZATION

Cornelius I. Weber

1970s SHIFT FROM BIOLOGICAL MONITORING TO TOXICITY

1975-ERL-Duluth, Charles Stephan publishes EPA report on:

"Methods for acute toxicity with fish, macroinvertebrates, and amphibians."

Based on ASTM committees E-35 & D-19

1978 - EMSL-Cincinnati, Cornelius Weber publishes EPA manual on:

"Methods for measuring the acute toxicity of effluents and surface waters."

Based on modifications to 1975 document
Expertise and capability of EPA Regional Labs
Emerging Agency QA guidance

1979 - EMSL-Cincinnati Publishes manual on:

"Handbook for analytical quality control in water and wastewater laboratories."

HISTORICAL EPA METHODS STANDARDIZATION

1980s - METHODS EMPHASIS SHIFTS TO CHRONIC TOXICITY

1983 - ORD EMSL-Cincinnati Publishes:

"Guidelines and format for EMSL-Cincinnati methods."

1984 - Office of Water Establishes Policy on:

Water Quality - Based approach to NPDES Permits

1985 - Office of Water Publishes:

"Technical support document for water quality-based toxics control"

1985 - EMSL-Cincinnati - Publishes manual on:

"Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms."

Based on ERL-Duluth peer review methods, EPA reports and capabilities of EPA biologists working in Environmental Service Division laboratories

HISTORICAL EPA METHODS STANDARDIZATION

1980s - CONTINUED

1988 - EMSL-Cincinnati - Publishes manual on:

"Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms."

Based on ERL-Naragannsett peer review methods, EPA reports and capabilities of EPA biologists working in Environmental Service Division laboratories

304 (H) Process

SECTION 304 (H) CLEAN WATER ACT - REQUIRES EPA

To promulgate guidelines establishing test procedures for the analysis of pollutants that shall include factors which must be provided in any certification pursuant to section 401 or permit application pursuant to section 402

1988 - 40 CFR Part 136

Guidelines Establishing Test Procedures for the analysis of pollutants

Applicability: to measurements performed for NPDES permit application reports required for NPDES permits i.e. DMRQA other enforcement actions

NPDES permit certification

other quantitative or qualitative effluent data

304 (H) PROCESS & TOXICITY TEST MANUALS REVISION

LATE 1980s SHIFT FROM GUIDELINE TYPE METHODS TO LEGALLY DEFENSIBLE METHODS

12/1989 EPA proposed rules on toxicity testing under 40 CFR Part 136 & requests comments

2/90 Public Comments complete

1990: 304 (H) subgroups formed to address comments

- toxicity methods
- statistics
- ames tests
- collection methods for viruses

Manuals revised based on Public and BAC comments

Submitted to BAC for final review

304 (H) PROCESS & TOXICITY TEST MANUALS REVISION

1991: Final Rule submitted for Red Border review

OGC non-concurred -

revisions to responses to public comments, rule & manuals, requires manuals to be published before rule.

OW concurred -

hold on rule until manuals published

Revised Acute toxicity test manual concurred on by OW and published.

1992: OW non-concurrs on 2 chronic manuals with 32 pages of comments.

ORD accepts most comments except for those technically not consistent with BAC consensus or those technically, scientifically, or statistically unsubstantiated.

EPA BIOLOGICAL ADVISORY COMMITTEE
1990s

ROLE HAS SHIFTED FROM ADVISORY COMMITTEE TO A VOTING CONSENSUS COMMITTEE

1991 BAC WAS RECHARTERED

STEERING COMMITTEE DREW UP CHARTER AND BAC REVIEWED AND APPROVED BAC MISSION, DUTIES, ORGANIZATION, SUBCOMMITTEES, FUNCTIONAL STATEMENTS OF SUBCOMMITTEES AND REPRESENTATION

BAC MISSION

PROVIDE TECHNICAL ADVICE TO THE AGENCY ON ALL BIOLOGICAL METHODS AND RELATED ECOLOGICAL ISSUES

BAC DUTIES

REVIEW, COMMENT, ASSIST (REGIONS, ORD, & PROGRAMS) AND VOTE ON TECHNICAL ISSUES DEALING WITH STANDARDIZING AND EVALUATING EPA BIOLOGICAL METHODS AND INDICATORS

EPA BIOLOGICAL ADVISORY COMMITTEE
1990s

BAC DUTIES

1. REVIEW, COMMENT, ASSIST (REGIONS, ORD, & PROGRAMS) AND VOTE ON TECHNICAL ISSUES DEALING WITH STANDARDIZING AND EVALUATING EPA BIOLOGICAL METHODS AND INDICATORS.

2. ENSURE STATES DEVELOP CONSISTENT & ECOLOGICALLY MEANINGFUL BIOLOGICAL CRITERIA THAT FACILITATES INTERSTATE, INTERREGIONAL, AND EMAP NATIONAL COMPARISONS.

3. EXCHANGE TECHNICAL INFORMATION AND EXPERIENCES ON BIOLOGICAL METHODS AND INDICATORS.

4. REVIEW, COMMENT, & ASSIST AGENCY BIOLOGICAL MONITORING OR BIOCRITERIA POLICY.

5. REPRESENT AGENCY ON BIOLOGICAL METHODS AND RELATED ISSUES ON NATIONAL EPA AND OTHER FEDERAL AGENCY COMMITTEES. i.e., EMMC, ITFMWQ

EPA BIOLOGICAL ADVISORY COMMITTEE
1990s

WHOSE ON BAC 1-2 BIOLOGISTS FROM REGIONAL OFFICES, PROGRAM OFFICES, AND ORD

ORGANIZATIONS REPRESENTED ON BAC

ORD

ERL-DULUTH
ERL-NARRAGANNSETT
ERL-CORVALLIS
ERL-GULF BREEZE
EMSL-CINCINNATI
EMSL-LAS VEGAS

REGIONS 1-10

OFFICE OF WASTEWATER ENFORCEMENT & PERMITS
OFFICE OF SCIENCE AND TECHNOLOGY
OFFICE OF WETLANDS, OCEANS, AND WATERSHEDS
OFFICE OF SOLID WASTE AND EMERGENCY RESPONSE
OFFICE OF POLLUTION PREVENTION

NATIONAL ENFORCEMENT INVESTIGATION CENTER

EPA BIOLOGICAL ADVISORY COMMITTEE
1990s

BAC ORGANIZATIONAL STRUCTURE

CHAIRPERSON
DEPUTY CHAIRPERSON

BAC STEERING COMMITTEE

TOXICITY ASSESSMENT SUBCOMMITTEE

CHAIRPERSON
VICE-CHAIRPERSON

STEERING SUBCOMMITTEE

ECOLOGICAL ASSESSMENT SUBCOMMITTEE

CHAIRPERSON
VICE-CHAIRPERSON

STEERING SUBCOMMITTEE

PRESENT ORD GUIDANCE ON METHODS STANDARDIZATION/VALIDATION

ORD CONSENSUS DOCUMENT OR WHITE PAPER

"1987 GUIDELINES FOR SELECTION AND VALIDATION OF U.S. EPA MEASUREMENT METHODS"

ORIGINAL INTENT WAS CHEMISTRY METHODS

BAC IS USING IT AS A FRAME FOR BIOLOGICAL METHODS

PURPOSE:

Provide a process of monitoring and regulatory methods selection, standardization, and validation.

PRESENT ORD GUIDANCE ON METHODS STANDARDIZATION/VALIDATION

SUMMARY OF GUIDELINES: SIX STEPS

1. DETERMINATION OF METHOD REQUIREMENTS AND DQOs	PROVIDED BY PROGRAM & REGIONAL OFFICES
2. METHOD SELECTION & DEVELOPMENT	PROGRAM & REGIONS
3. SINGLE-LAB EVALUATION	SENSITIVITY OF METHOD VARIABLES (RUGGEDNESS) PRECISION SINGLE-OPERATOR SINGLE-LABORATORY

PRESENT ORD GUIDANCE ON METHODS STANDARDIZATION/VALIDATION

SUMMARY OF GUIDELINES: CONTINUED

4. CONFIRMATORY TESTING	EVALUATION OF A MINIMUM OF 3 LABS
5. INTERIM METHOD DESCRIPTION	FULL METHOD RUGGEDNESS TESTED
	MANDATORY & OPTIONAL TEST CONDITIONS
	GUIDANCE ON DATA ANALYSES
	SINGLE-LAB PRECISION
	ENDORSED BY AGENCY "STANDARD" METHOD
6. FORMAL COLLABORATIVE (INTERLAB STUDY)	COMPLETE ACCEPTABLE DATA FROM A MINIMUM OF SIX LABS

EMMC BIOLOGICAL METHODS INTEGRATION WORKGROUP

5/92 REQUEST FROM EMMC TO BAC TO FORM NEW WORKGROUP ON BIOLOGICAL METHODS

8/92 BALLOT FOR TWO CO-CHAIRS

ONE FROM REGIONAL LABORATORY

ONE FROM PROGRAM OFFICE

BAC CHAIRMAN IS THIRD CO-CHAIR

REPRESENTING OMMSQA - ORD

FY 93 EMMC IS CONSIDERING TAKING OVER COMPENDIUM OF BIOLOGICAL METHODS STARTED BY OTTRS

INTENT TO PUT IN EMMI & USE COMPENDIUM TO INITIATE METHODS INTEGRATION

EMMC Methods Format

William Telliard, U.S. EPA Office of Science and Technology

I. **Scope and Application** - Tabular format whenever possible

- A. Analyte list
- B. CAS numbers
- C. Matrices
- D. Method sensitivity (expressed as mass and as concentration with a specific sample size)
- E. Data quality objectives

II. **Summary of Method**

III. **Definitions**

IV. **Interferences**

V. **Safety**

- A. Above and beyond good laboratory practices
- B. Disclaimer statement (look at ASTM disclaimer)
- C. Special precautions
- D. Specific toxicity of target analytes or reagents
- E. Not appropriate for general safety statements

VI. **Equipment and Supplies**

VII. **Reagents and Standards**

VIII. **Sample Collection, Preservation and Storage**

- A. Provides information on sample collection, preservation, shipment and storage conditions.
- B. If holding times are exceeded, data may have changed and should be flagged for data user's attention.

IX. **Quality Control**

- A. This section provides a summary of the QC requirements of the method.

X. **Calibration and Standardization**

A. Should include calibration steps that are not followed daily; daily calibration steps will be included in the procedure section.

XI. **Procedure**

XII. **Data Analysis and Calculations**

XIII. **Method Performance**

A. A precision/bias statement should be incorporated in the section, including detection limits, and source/limitations of data.

XIV. **Pollution Prevention**

A. Cite good laboratory practices for pollution prevention.

XV. **Waste Management**

A. Cite how waste and samples are properly disposed.

XVI. **References**

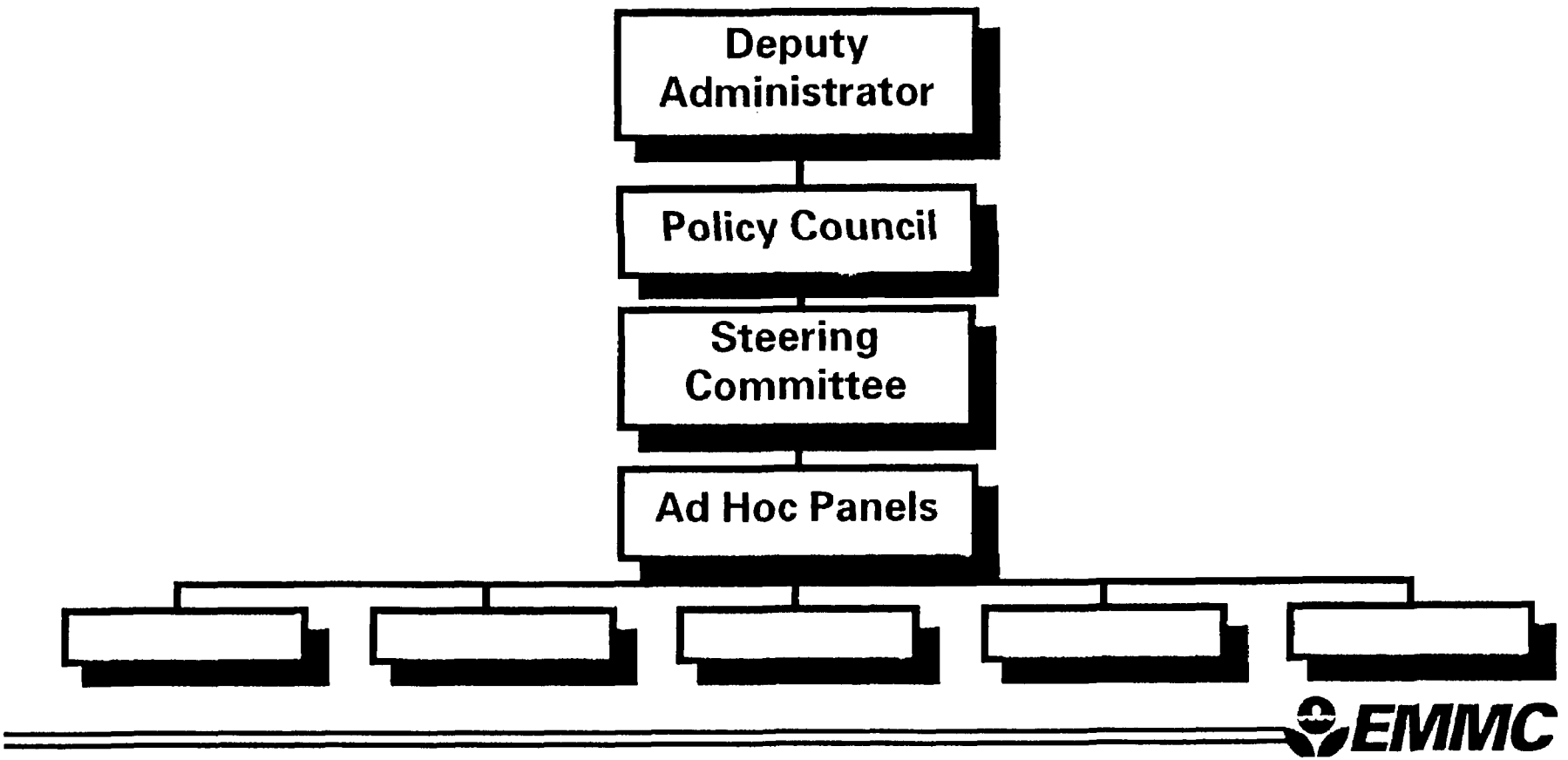
A. Source documents
B. Publications

XVII. **Tables, Diagrams, Flowcharts, and Validation Data**

A. Location of these items will be left to the judgement of the individual work group.

(Finalized as a result of January 24, 1992, balloting by Members of EMMC Methods Integration Panel and Work Group Tri-chairs.)

EMMC Organization



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Ad Hoc Panels

- Quality Assurance Services
- Methods Integration
- Automated Methods Compendium
- Analytical Methods & Regulation Development
- National Laboratory Accreditation

EMMC

A52-001-3

Methods Consolidation Work Groups

- Water Media Work Group
- Solid Media Work Group
- Air Media Work Group
- QA/QC Work Group

EMMC

A52-001-3

Integration of Existing Methods

Status of Pilot Methods
Five draft methods prepared and undergoing testing:

- Strong acid-conventional heating
- Strong acid-microwave heating
- ICP-AES
- Furnace AA
- Volatile organic analytes

EMMC

A52-001-3

Approach to Methods Integration

- Define DQO's
- Agree on method format (EMMC has a draft format)
- Agree on terminology (EMMC draft)
- Place all existing methods in consideration
- Compile data supporting each method in a single database

EMMC

A52-001-3

Work Group Priorities

Water Media Work Group:

- Pesticides
- Chlorinated dioxins and furans
- Non-freon methods for oil and grease
- Definitions

Work Group Priorities (Cont'd)

Solids Media Work Group:

- Semi-volatile organics

EMMC

A52-001-3

Work Group Priorities (Cont'd)

Air Media Work Group

- Information gathering
- Problems/priorities identification with Regions

EMMC

A52-001-3

Work Group Priorities (Cont'd)

QA/QC Work Group:

- Validation issues
  - Level of validation guidelines
  - Validation procedures
- Unification of QC requirements
- Unification of definitions
- Definition of method performance

EMMC

A52-001-3

Performance-Based Methods vs. Control-Based Methods

- Definable method specifications
- Flexible specifications are necessary to obtain the desired DQO through direct substitution
- In a directed method, the performance is not necessarily known and thereby requires the step-by-step procedure

The Environmental Monitoring Methods Index

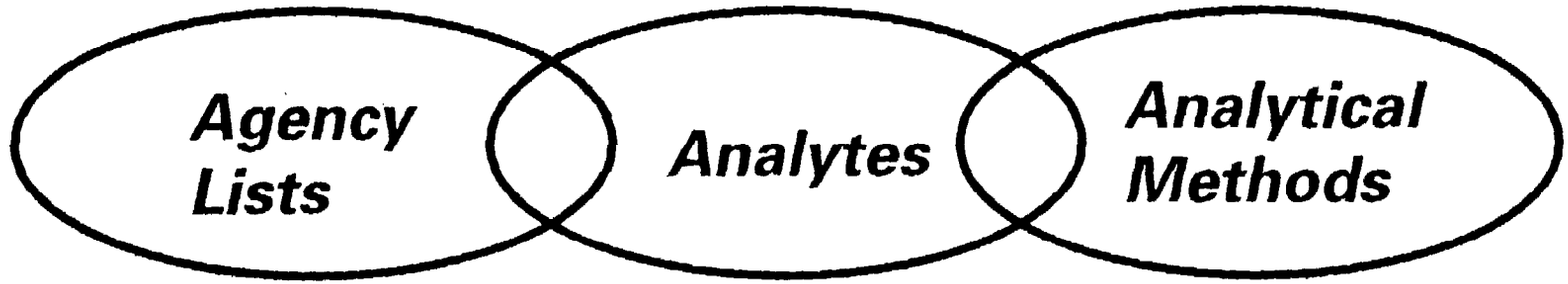
- EPA's official Methods database linking 50 EPA regulatory lists, 2,600 substances and 926 analytical methods

EMMC

A52-001-3

What Is The Environmental Monitoring Methods Index?

- The Environmental Monitoring Methods Index is a comprehensive cross-referencing tool for information on:



EMMC

A52-001-3

Freshwater Sediment Toxicity Assays: Necessary and Desirable Attributes

G. Allen Burton, Jr., Wright State University, Department of Biological Sciences - Dayton, OH

I. What's Available?

II. Assay Strengths/Weaknesses

III. Assay Requirements

A. Necessary attributes (from a scientific perspective)
B. Desirable attributes (from a regulatory/program/project perspective)

IV. Examples of Assay Evaluations

V. Preliminary Recommendations

Toxicity Testing

usefulness improving rapidly

- method refinement
- short-term chronic assays
- toxicant interactions simplified
- reduced uncertainty

Selected Freshwater Sediment Toxicity Tests

Organism Group	Response Measures	Test Species
Amphibians	Embryo-larval survival, Terata	*Xenopus laevis*
Fish	Embryo-larval survival, Length Weight, Terata	*Pimephales promelas* *Oncornynchus mykiss* *Oryzius latipes*
Zooplankton	Survival, Reproduction	*Daphnia magna* *Ceriodaphnia dubia* *Brachionus* sp. *Colpidium campylum*
Benthic Invertebrates	Survival, Size, Reproduction, Molting, Emergence, Avoidance	*Panagrellus redivivus* *Caenorhabditis elegans* *Tubifex tubifex* *Stylodrilus heringianus* *Pristina leidyi* *Lumbriculus variegatus* *Hyalella azteca* *Diporeia sp.* *Gammarus pulex* *Gammarus fasciatus* *Corbicula fluminea* *Anodonta imbecillis* *Chironomus tentans* *Chironomus riparius* *Hexagenia limbata* *Hexagenia bilieata*
Microbial	Luminescence (Microtox™)	*Photobacterium phosphoreum*
Phytoplankton	Cell number, ^{14}C uptake	*Selenastrum capricornutum*
Macrophytes	Frond number, chlorophyll, biomass, root and shoot length, peroxidase	*Lemna* sp. *Hydrilla verticillata*
Benthic Indigenous Communities	Structure indices, functional indices, chlorophyll, respiration, enzyme activities	Bacteria Protozoan Periphyton Phytoplankton Macroinvertebrate

Optimal Toxicity Assay Issues

Validation
- Relevance
- Sensitivity/discriminatory
- Exposure design
- Response dynamics

Resources
- Organism availability
- Laboratory resources
- Expertise
- Expense, time

Standardization
- Methods
- QA/QC criteria
- Adequate database

Selection of Optimal Assessment Endpoints

Project objectives

Site characteristics

Available methods

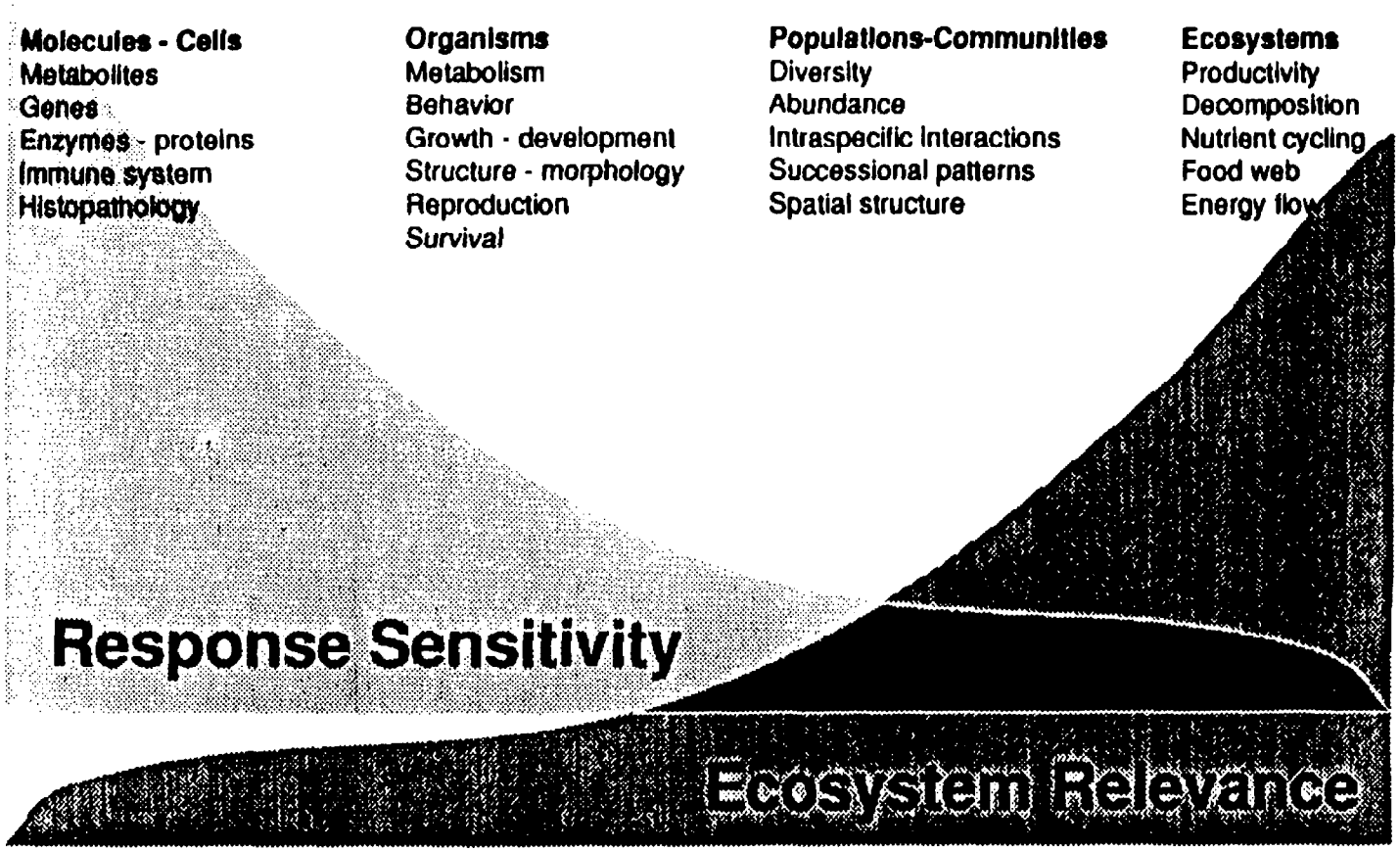
Key components represented

Assay Sensitivity: effect vs. control or reference

Realistic protection of study ecosystem

(i.e., nationwide, ecoregion, site-specific)

- Relevance
- Validity
- Significance

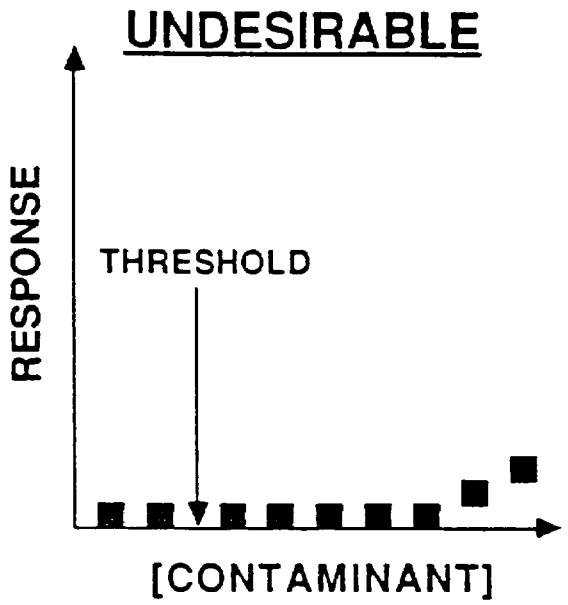

Molecules - Cells
Metabolites
Genes
Enzymes - proteins
Immune system
Histopathology
Organisms
Metabolism
Behavior
Growth - development
Structure - morphology
Reproduction
Survival
Populations-Communities
Diversity
Abundance
Intraspecific interactions
Successional patterns
Spatial structure
Ecosystems
Productivity
Decomposition
Nutrient cycling
Food web
Energy flow
Response Sensitivity
Ecosystem Relevance
(seconds - days)
(minutes - years)
(days - years)
(weeks - decades)
Response Time

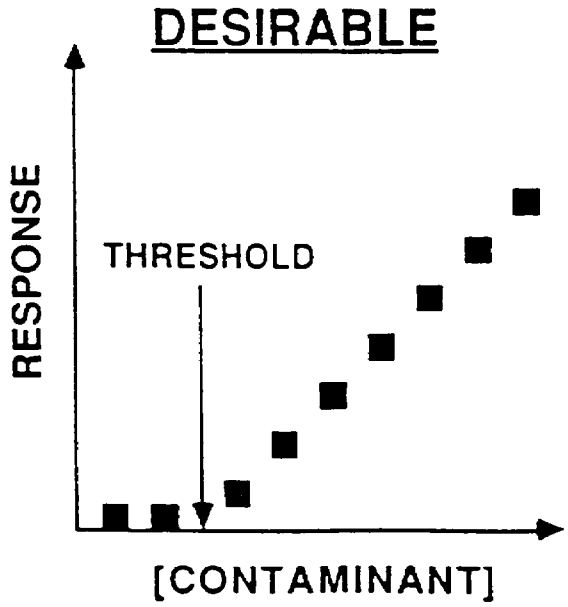
(Burton 1991)

Factors affecting sensitivity

- Measured response
- Organism type
- Life stage
- Health
- Test design (e.g., exposure period)
- Sample manipulation

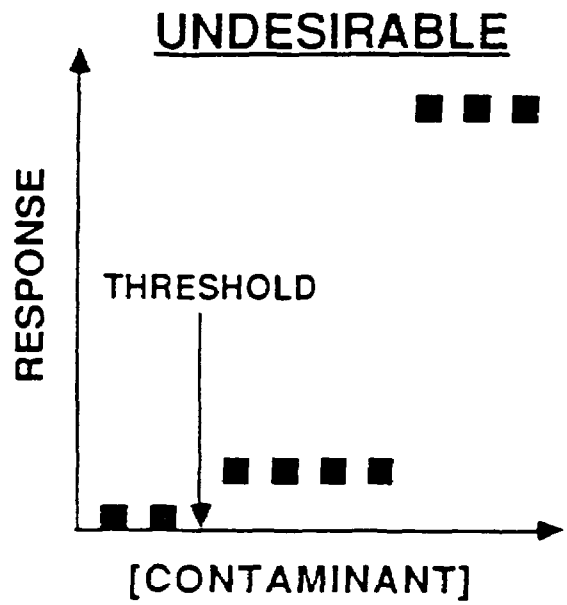
SENSITIVITY

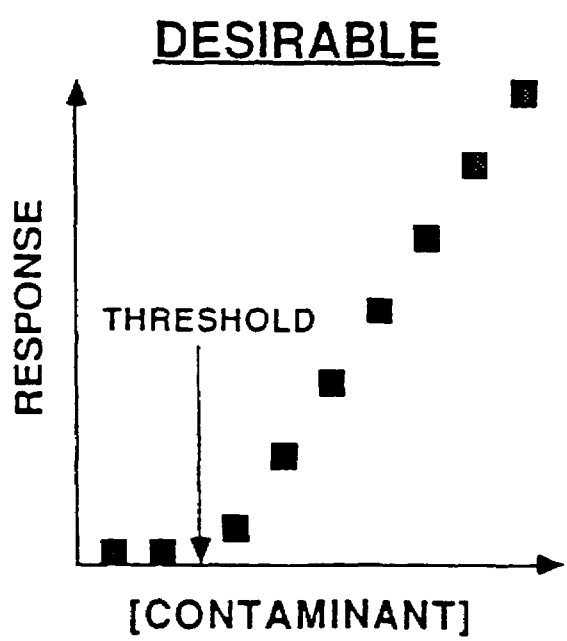




(Ross et al. 1991)

DISCRIMINATION





(Ross et al. 1991)

Necessary Attributes

- Sensitive (responsive)
- Discriminates (discerns degree of contamination)
- Relevant to ecosystem/study objectives
  (Species *and* exposure design)
- Validity (field verified, few false +/-)

Desirable Attributes

A. Agency Specific:

- Comprehensive indicator
- Reliable
- Resource requirements
- Standardized

B. Additional Program/Project Specific:

- Uniqueness (non-redundant)
- Confirmatory (weight-of-evidence)
- Significance (ecosystem, commercial, societal)

What Makes An Assay Relevant?

Consider the:

Test ecosystems' characteristics

Sample manipulation artifacts

Organisms' route of exposure(s)

Organisms' ecological niche

Measured response sensitivity
(e.g., mortality vs. reproduction)

Organism stress

For example:

Trout $\neq$ Carp

Hyalella $\neq$ Aquatic worms (e.g., Tubifex)

Elutriate $\neq$ Solid phase

Pore $\sim$ Solid phase ?

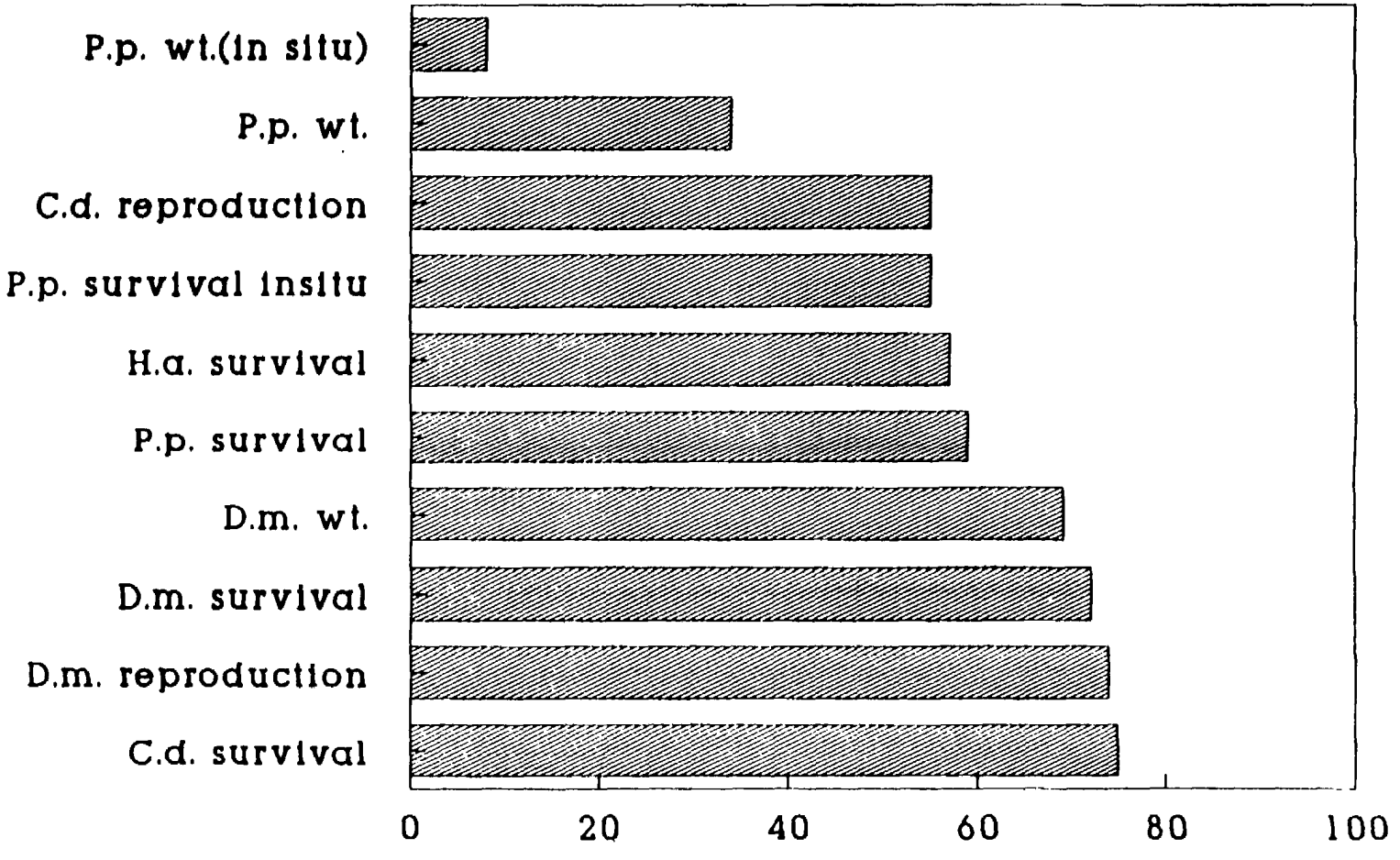
Benthos $\sim$ Nonbenthos ?

Lab response $\sim$ In situ response ?

Total Quality Assurance

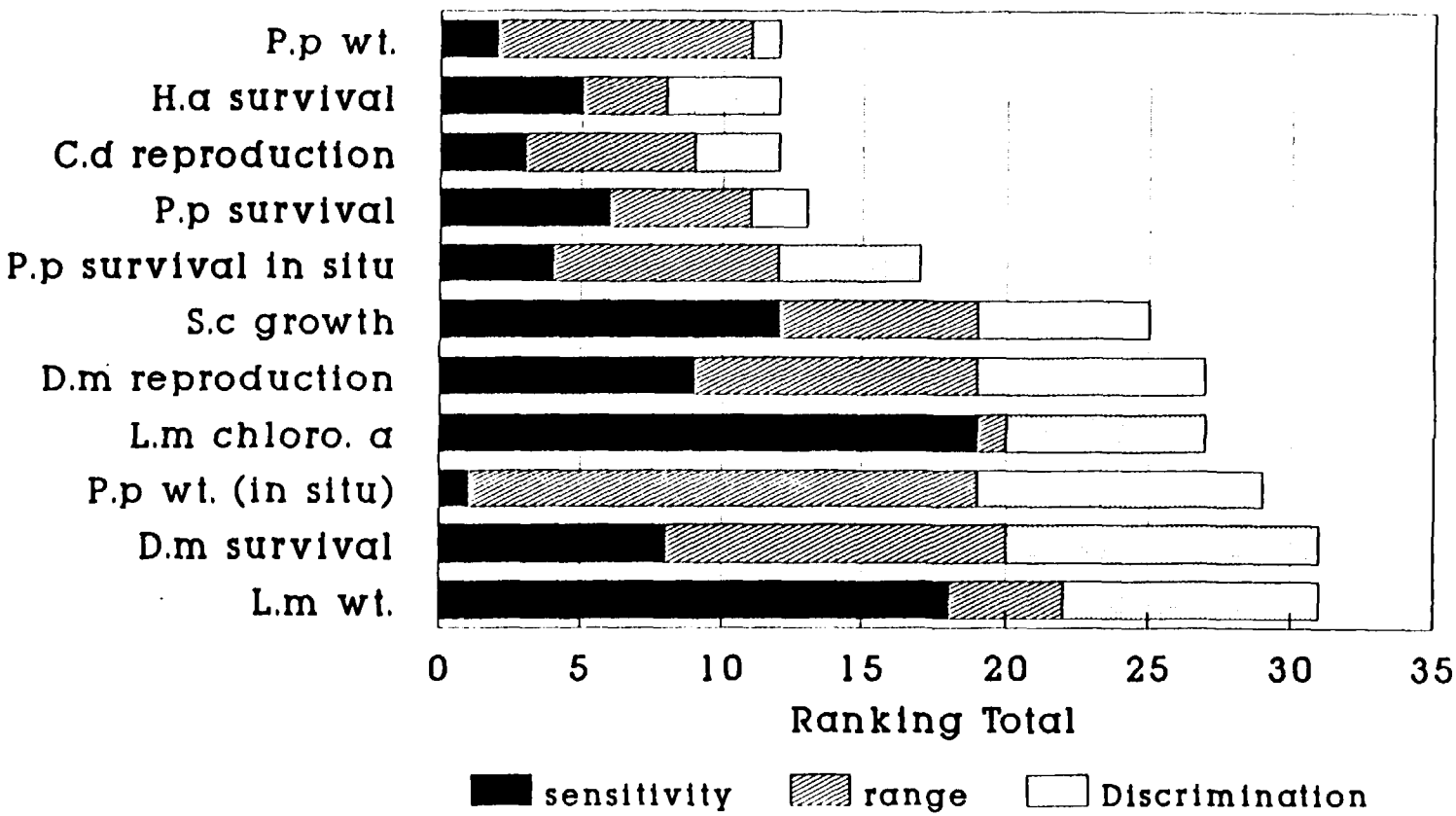
- Study design
- Sample collection/manipulation
- Exposure design
- Assay performance criteria

Little Scioto River
7 Day Response ($\bar{x}$%)

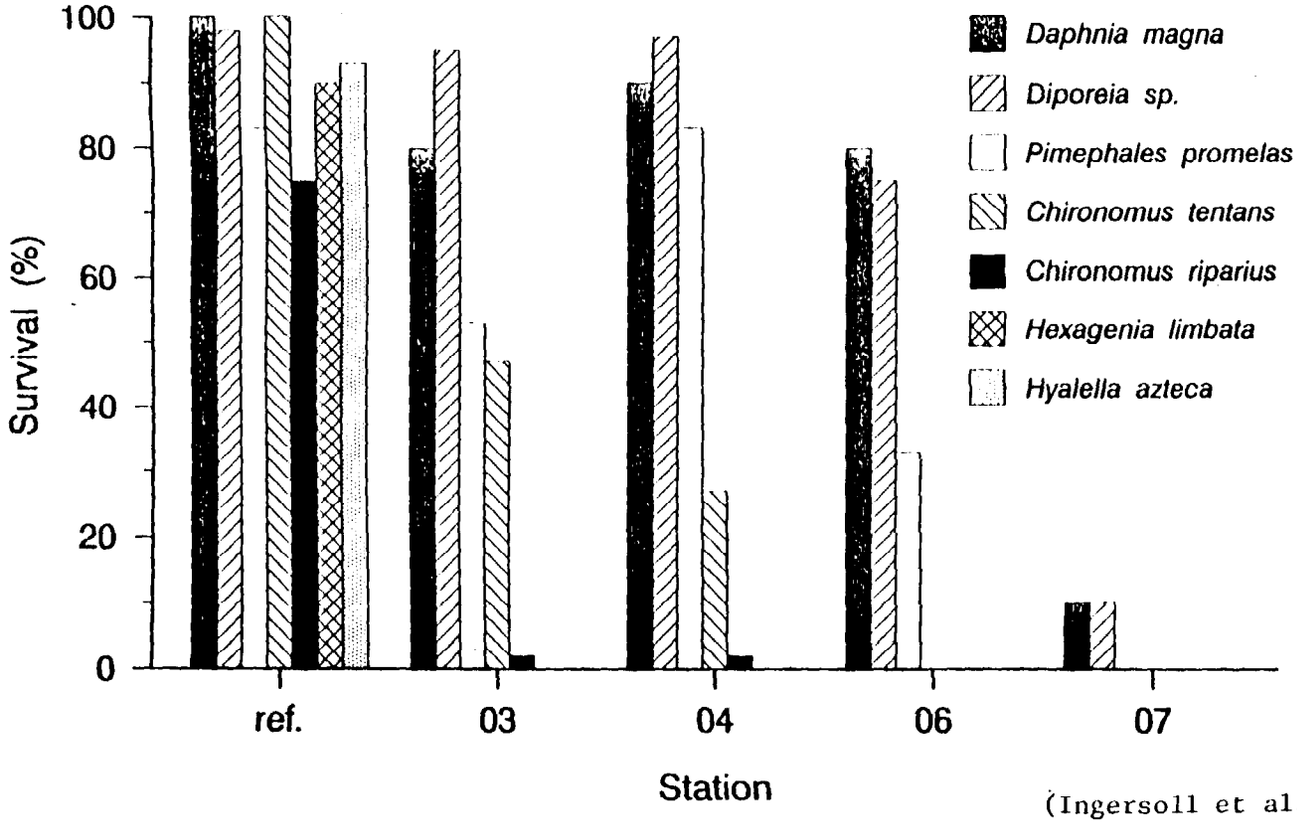


Little Scioto River (Oct.1990)

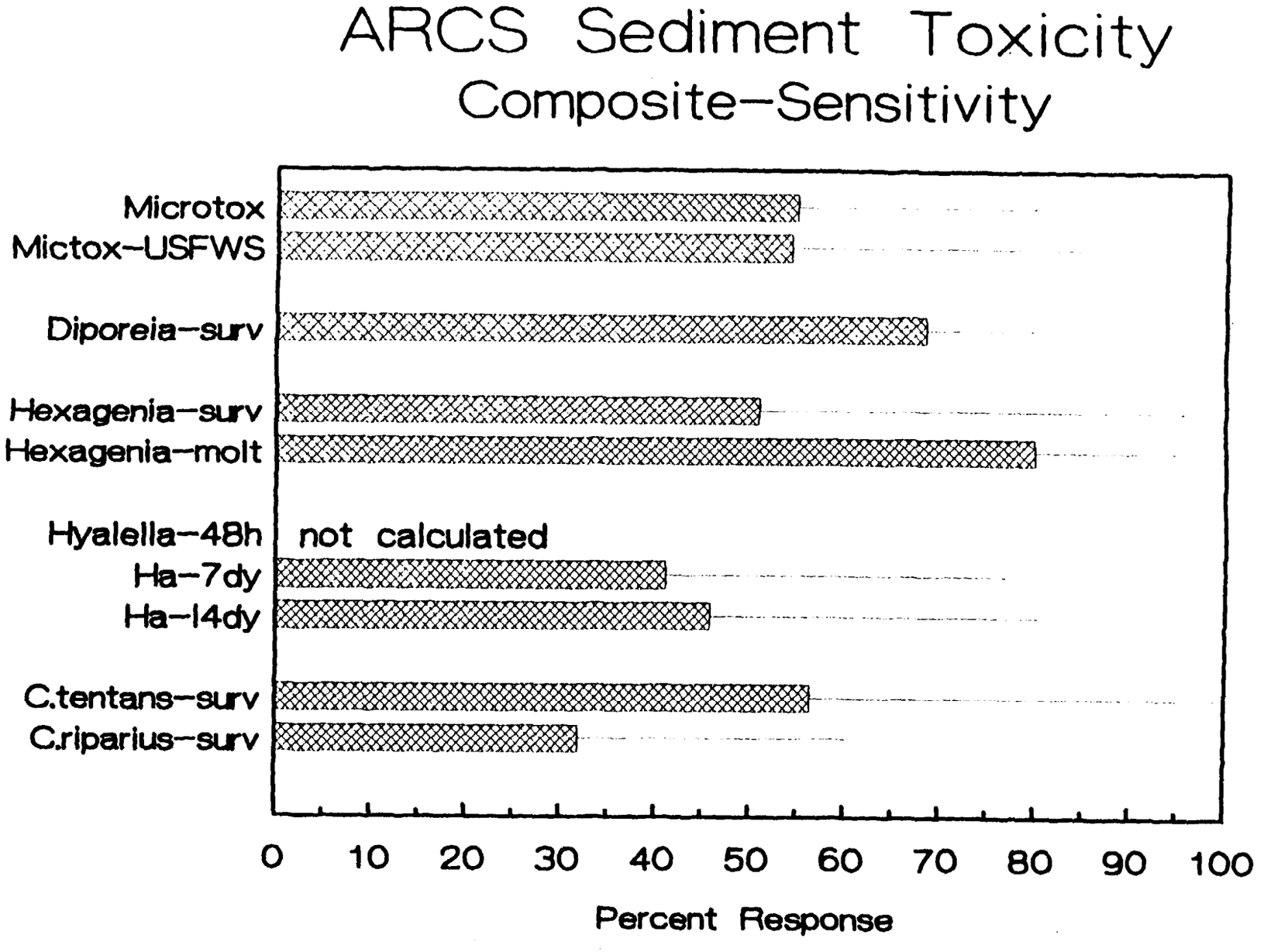
Composite Ranking



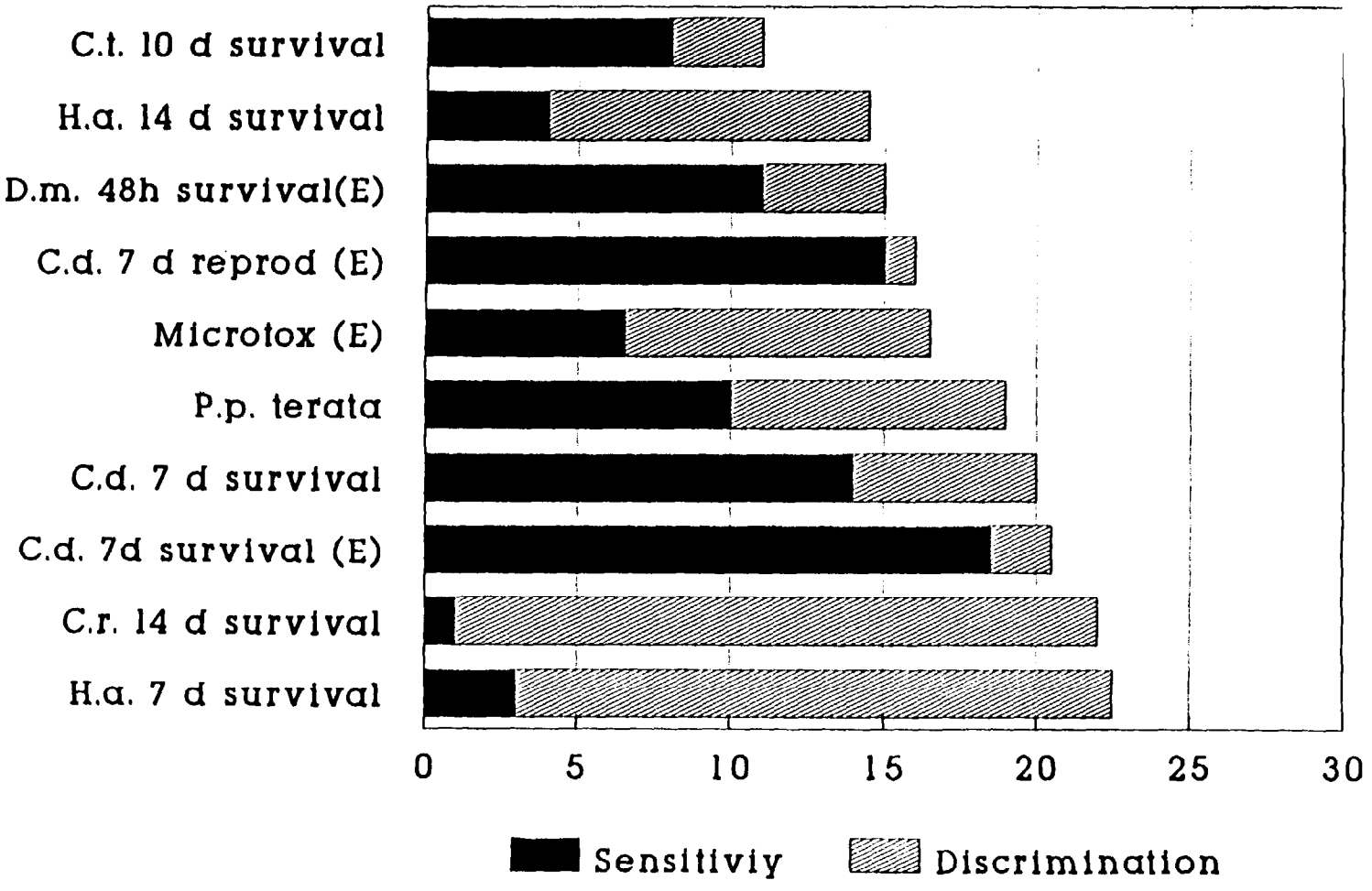
Whole Sediment Toxicity Tests
Indiana Harbor



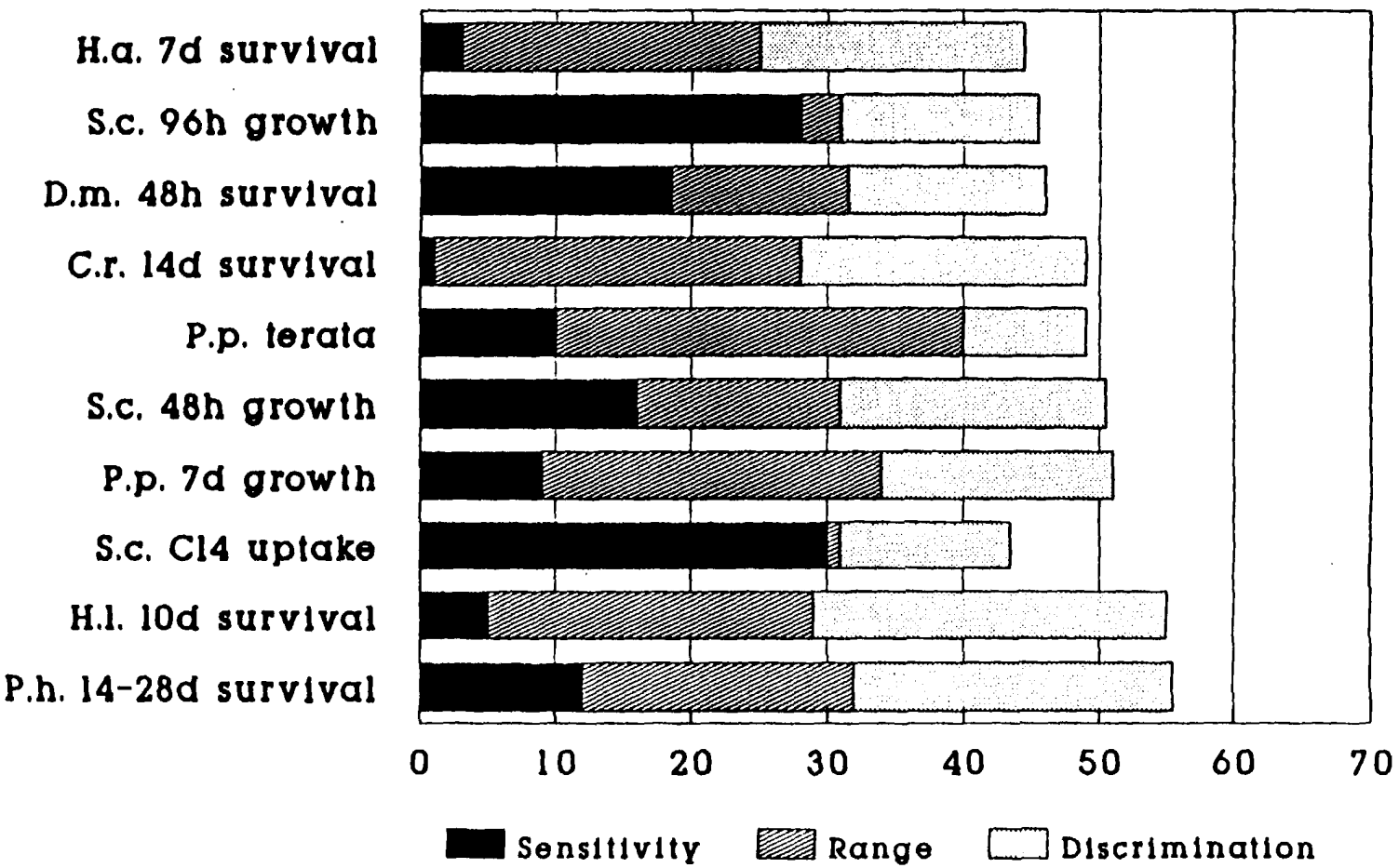
(Ingersoll et al. 1990)


ARCS Sediment Toxicity
Composite-Sensitivity
Microtox
Mictox-USFWS
Diporeia-surv
Hexagenia-surv
Hexagenia-molt
Hyalella-48h
not calculated
Ha-7dy
Ha-14dy
C.tentans-surv
C.riparius-surv
0
10
20
30
40
50
60
70
80
90
100
Percent Response

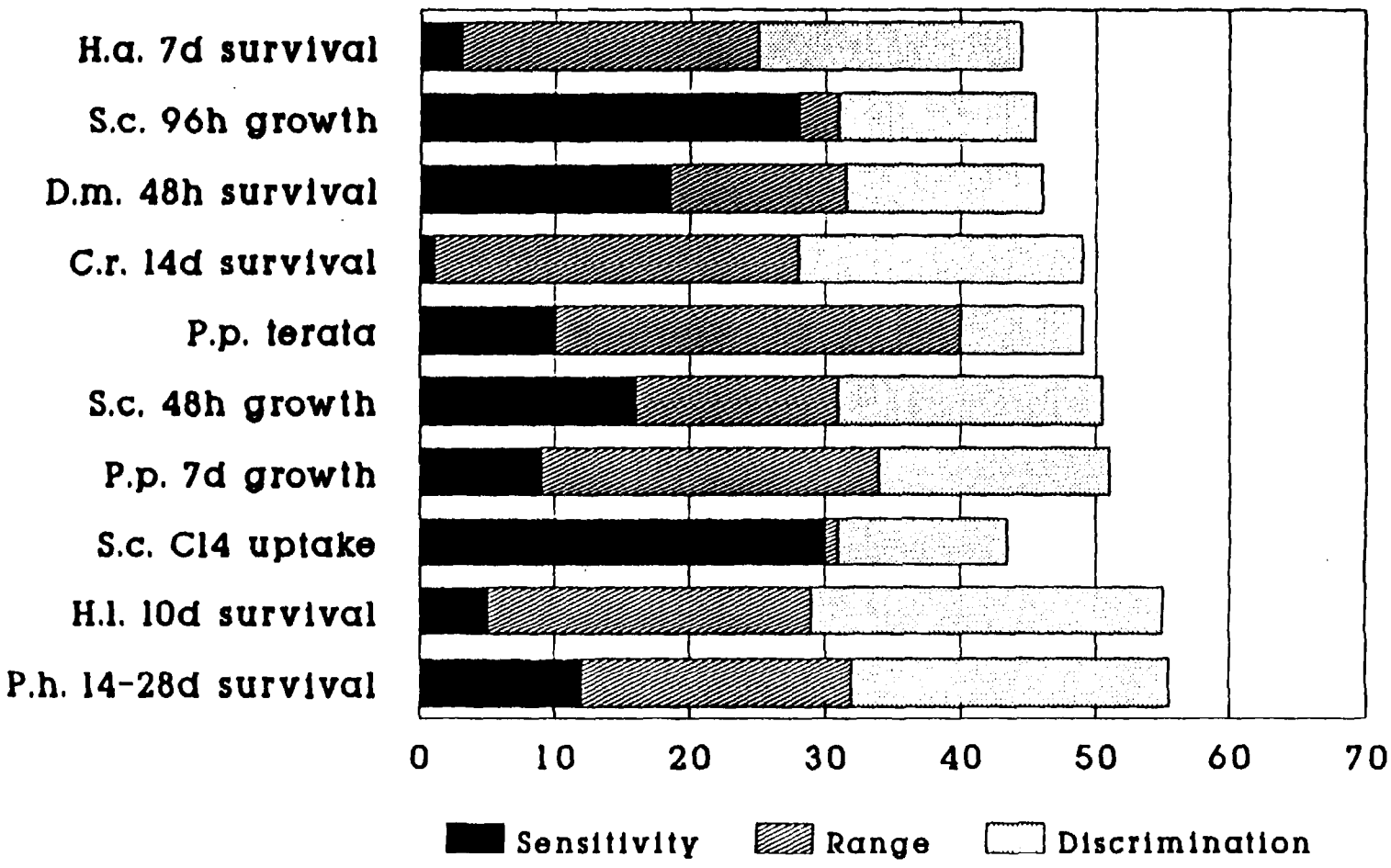
Ranking- Sensitivity + Discrimination



Ranking Components



Ranking Components



Principal Components Factor Analysis: ARCS Assay Comparison

Factor 1	Factor 2	Factor 3	Factor 4
P. promelas (wt.)	D. magna (survival)	Hexagenia (survival)	Hexagenia (molting)
H. azteca (survival)	C. dubia (survival)	C. dubia (young)	
D. magna (young)			
P. promelas (terata)			
Variance explained			
40%	23%	13%	11%

Summary Ranking
(Ingersoll et al., 1992)

	Protectiveness	Similarity	Sum
H. azteca 14-D	1	3	4
H. azteca 28-D	2	5	7
C. riparius 14-D	3	1	4
Microtox	4	2	6
D. magna	5	4	9
C. tentans 10-D	6	6	12

Sediment Toxicity Sensitivity Comparisons

- Twelve studies by Ankley, Burton, Cairns, Giesy, Hoke, Ross, et al.
- Comparisons of 3 to 20 assays/study

Most sensitive assays

Group A: Hyalella azteca 7-14 d survival

Chironomus riparius 7 d survival

Daphnia magna 2-7 d survival

Group B: Ceriodaphnia dubia 7 d reproduction

Pimephales promelas 7 d larval growth

Chironomus tentans 10 d growth

A = Most sensitive in at least 2 studies
B = Second and third in sensitivity in at least 3 studies

Test Battery Recommendations

	Burton et al.	Giesy-Hoke	IJC	IJC
Daphnia magna (48 h)		•		•
(7 d)	•		•	
	(or)			
Ceriodaphnia dubia (7 d)	•		•	
Hyalella azteca (7-14d)	•			
(28 d)	(or)			•
Chironomus riparius (10 d)	•			(or)
	(or)			
Chironomus tentans (10 d)	•	•		•
	(or)			(or)
Hexagenia limbata (10 d)	•			•
Microtox	•	•	•	•
Selenastrum capricornutum		•		
Algal fractionation bioassay			•	•
Ames			•	

General Purpose Short List

I. a. Microtox (screen in tandem)

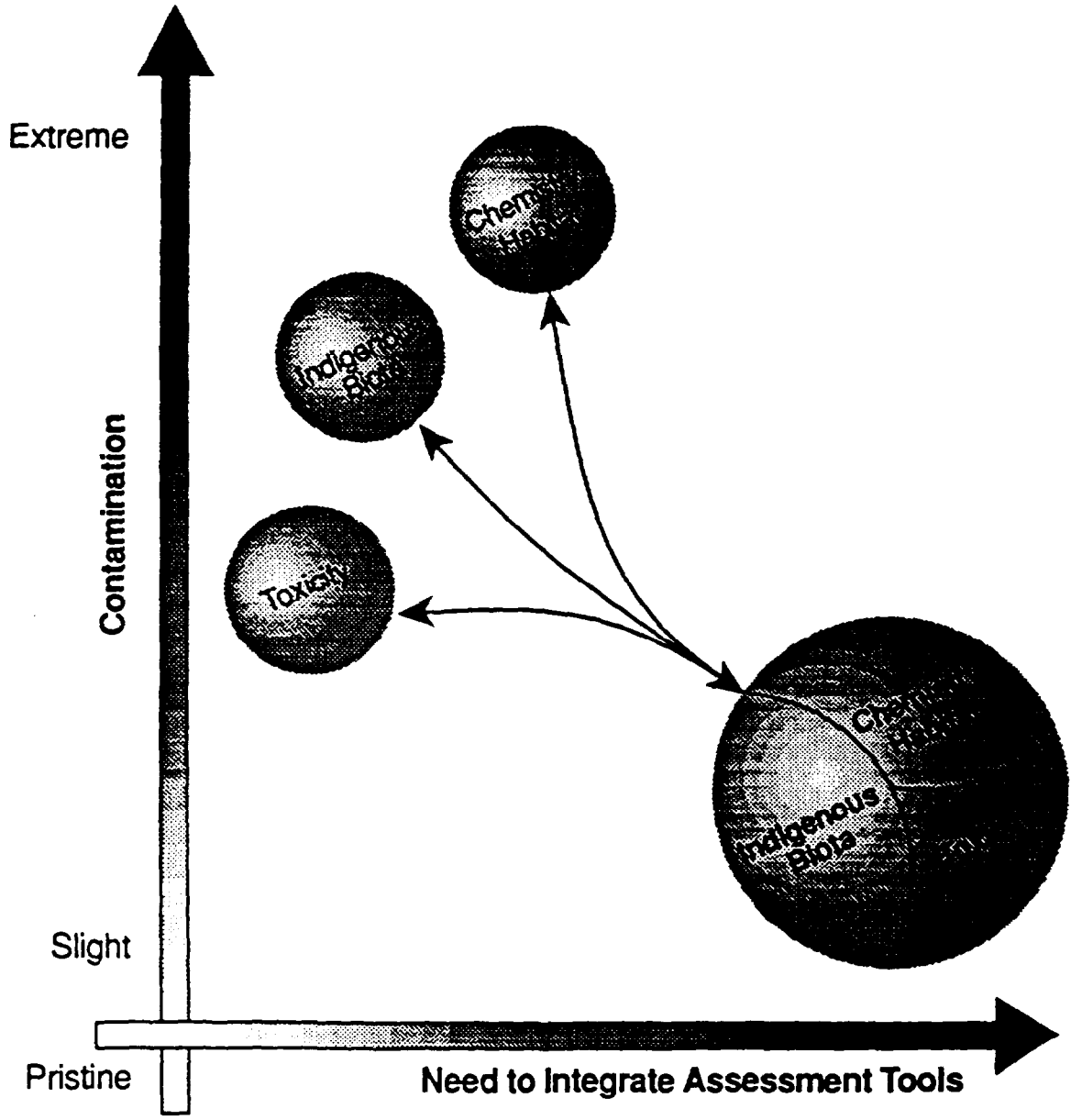
b. Ceriodaphnia dubia or Daphnia magna (3 brood)

c. Hyalella azteca, Chironomus tentans or C. riparus, or Hexagenia limbata (7-14 day)

II. a. Pimephales promelas (early life stage)

b. Selenastrum capricornutum

III. Other assays in tandem with above



Burton & Scott 1992)

Conclusions

- Consider **all** assessment issues
- Test multiple, relevant species
- Test multiple trophic levels
- Validate laboratory responses
- Use proven methods
- Expect site variance

Midge Whole Sediment Bioassays

John P. Giesy and Jody A. Kubitz, Michigan State University - East Lansing, MI

The aquatic midge Chironomus tentans has been effectively used as a bioassay organism to predict the toxicity of sediments to benthic organisms. This organism is easy to culture and maintain and standardized protocols exist to produce a continuous supply of known-aged individuals. The organisms are hardy and easy to manipulate in bioassays. Growth of C. tentans is a sensitive measure of response, which gives good discrimination power among sediments. Studies have been conducted to calibrate the response of the survival and growth studies to community structure, and some information on the relative sensitivity of the midges to chemicals is available.

There are several issues that need to be addressed if C. tentans assays are to be adopted for routine use. Theses include: more studies of the relative sensitivity of the midges to chemicals; more comparisons between laboratory and field studies; investigations of the genetic drift of laboratory cultures; and comparisons of partial life cycle tests with whole life tests for toxicants of several modes of action.

Chironomus tentans Advantages

- continuous supply of known-age individuals
- easy to culture - standardized
- size easy to manipulate & weigh
- associated with sediment

Giesy & Kubitz, 1992

Chironomus tentans Advantages II

- naturally occurring in ecosystems of interest
- much toxicological information
- short, predictable life span
- hardy - not easily damaged

Giesy & Kubitz, 1992

Chironomus tentans Advantages III

- discriminatory power in chronic tests
- statistical significance known - power tests related to community structure
- standardized tests available
- whole-life or partial-life tests
- sediment or no-sediment tests

Giesy & Kubitz, 1992

Chironomus tentans Disadvantages

- relatively insensitive to acute lethality (tolerant of metals) - sometimes good
- don't feed on sediments, eat resuspended particles on surface
- less acute & chronic data for use in Sediment Quality Criteria

Giesy & Kubitz, 1992

Chironomus tentans Disadvantages II

- genetic drift and lab-to-lab variation
- sporadic, unexplained loss of vigor in culture
- potential for loss (pupation or emergence) of adults

Giesy & Kubitz, 1992

Chironomus tentans Issues - I

- duration of test, when to start
- endpoints: growth, survival, reproduction of F1, enzymes, body burden
- volume of test sediment and water
- food/culture medium

Giesy & Kubitz, 1992

Chironomus tentans Issues - II

- water replacement - yes/no
- flow-through - yes/no
- aeration - yes/no
- homogenization sediment - yes/no
- sterilization of sediment - yes/no how?

Giesy & Kubitz, 1992

Chironomus tentans Research Needs

- more lab-to-field calibration
- more info. on vector of accumulation
- more relative toxicity information
- calibration of partial to whole-life tests
- most sensitive life stage
- inter-laboratory calibration

Giesy & Kubitz, 1992

Chironomus tentans Conclusions

- of all the available tests, this is one of the most well developed, calibrated and validated
- high probability of being able to conduct a valid test
- predictability of field conditions is good, but data are limited

Giesy & Kubitz, 1992

Desirable and Necessary Attributes for Freshwater Sediment Toxicity Tests: Hyalella azteca

Chris Ingersoll, U.S. Fish and Wildlife Service, NFCR - Columbia, MO

I. Objectives

A. Life history of Hyalella azteca
B. Culture and test methods
C. Research needs for standard development

II. Hyalella azteca Life History

A. Species: Hyalella azteca (Saussure; talitrid amphipod)
B. Habitat: lakes, ponds, streams
C. Distribution: North America and Caribbean
D. Salinity: Euryhaline; fresh water up to about 22 g/L, culture 10 to 15 g/L
E. Life stages: Immature (1st 5 instars), juvenile (6th & 7th instar), adult (8th instar and older; about 35 d at 20 degrees C)
F. Growth: Indeterminant; male larger than female; male enlarged gnathopods
G. Feeding: Omnivore; bacteria and algae < 65 um
H. Behavior: Epibenthic, burrow in sediment w/o vegetation

III. Hyalella azteca Culture Methods

A. Flow: Static renewal, or flow through
B. Temperature: 20 to 25 degrees C
C. Light: 16:8 photoperiod; 50 to 100 ft. candles
D. Chamber: 1 L to 100 L
E. Age of animals: Known age vs. mixed age
F. Water quality: Soft water (ERL-Duluth) vs. hard water (ERL-Corvalis) strain
G. Aeration: Moderate
H. Feeding: Maple leaves, Tetramin, Rabbit Chow, diatoms
I. Substrate: Maple leaves, Mitex screen, cotton gauze, 3-M base web plastic

IV. Hyalella azteca Test Methods

A. Flow: Static, renewal, flow through
B. Temperature: 20 to 25 degrees C
C. Light: 16:8 photoperiod; 25 to 50 ft. candles
D. Chamber: 25 mL, 1 L, up to 100 L
E. Sediment ration: 1:1 to 1:4 ratio sediment: water
F. Age of animals: Known age (0 to 7 d, 7 to 14 d) vs. mixed age (size about 7 to 14 d)
G. No. animals: 5 to 20/beaker; 4 to 5 replicates/treatment
H. Duration: 7 d, 10 d, 14 d, 28 d
I. Endpoints: Survival, length, weight, sexual maturation (males), young production
J. Water Quality: Soft water (ERL-Duluth) vs. hard water (ERL-Corvallis) strain
K. Aeration: None or moderate
L. Feeding: None, Rabbit Chow, YCT, maple leaves, Tetramin
M. Acceptability: survival (80%), length, weight
N. Particle size: low sensitivity (with sufficient food)?
O. NH_3 and H_2S: low to moderate sensitivity?
P. Sediment contact: Mayflies = Midges > Amphipods > Daphnids
Q. Sensitivity: Daphnids > Amphipods = Mayflies > Midges
R. Reliability: Amphipods = Daphnids > Midges > Mayflies

V. Research Needs for Standardization of Hyalella azteca

A. Culture and testing: know-age and feeding
B. Culture and testing: reconstituted water
C. Reconstituted sediment
D. Abiotic factors
E. Reference toxicants
F. Species and strain sensitivity
G. Inter-laboratory comparisons
H. Life history and chronic indicators of toxicity
I. Spiking methods and positive controls
J. Dilution studies and mixtures
K. Laboratory to in situ comparisons

OBJECTIVES

- Life history of Hyalella azteca
- Culture and Test Methods
- Research Needs for Standard Development

HYALELLA AZTECA LIFE HISTORY

Species:	Hyalella azteca (Saussure; talitrid amphipod)
Habitat:	lakes, ponds, streams
Distribution:	North America and Caribbean
Salinity:	Euryhaline; fresh water up to about 22 g/L
	Culture 10 to 15 g/L

HYALELLA AZTECA LIFE HISTORY (cont.)

Life stages:	Immature (1st 5 instars)
	Juvenile (6th and 7th instar)
	Adult (8th instar and older; about 35 d at 20°C)
Growth:	Indeterminant; male larger than female; male enlarged gnathopods
Feeding:	Omnivore; bacteria and algae < 65 um
Behavior:	Epibenthic; burrow in sediment w/o vegetation

HYALELLA AZTECA CULTURE METHODS

Flow:	Static, Renewal, or flow through
Temperature:	20 to 25°C
Light:	16:8 photoperiod; 50 to 100 ft. candles
Chamber:	1 L to 100 L

HYALELLA AZTECA CULTURE METHODS (cont.)

Age of Animals:	Known age vs. mixed age
Water Quality:	Soft water (ERL-Duluth) vs. hard water (ERL-Corvallis) strain
Aeration:	Moderate
Feeding:	Maple leaves, Tetramin, Rabbit Chow, diatoms
Substrate:	Maple leaves, Nitex screen, cotton gauze, 3-M base web plastic

HYALELLA AZTECA TEST METHODS

Flow:	Static, Renewal, flow through
Temperature:	20 to 25°C
Light:	16:8 photoperiod; 25 to 50 ft. candles
Chamber:	25 mL, 1 L, up to 100 L
Sediment ratio:	1:1 to 1:4 ratio sediment: water
Age of animals:	Known age (0 to 7 d, 7 to 14 d)
	vs. mixed age (size about 7 to 14 d)
No. animals:	5 to 20/beaker; 4 to 5 replicates/treatment

HYALELLA AZTECA TEST METHODS (cont.)

Duration:	7 d, 10 d, 14 d, 28 d
Endpoints:	Survival, length, weight, sexual maturation (males), young production
Water Quality:	Soft water (ERL-Duluth) vs. hard water (ERL-Corvallis) strain
Aeration:	None or moderate
Feeding:	None, Rabbit Chow, YCT, maple leaves, Tetramin
Acceptability:	Survival (80%), length, weight

HYALELLA AZTECA TEST METHODS (cont.)

Particle size: Low sensitivity (with sufficient food)?

NH_3 and H_2S: Low to moderate sensitivity?

Sediment contact: Mayflies = Midges > Amphipods > Daphnids

Sensitivity: Daphnids > Amphipods = Mayflies > Midges

Reliability: Amphipods = Daphnids > Midges > Mayflies

RESEARCH NEEDS FOR STANDARDIZATION OF HYALELLA AZTECA

- CULTURE AND TESTING: KNOWN-AGE AND FEEDING
- CULTURE AND TESTING: RECONSTITUTED WATER
- RECONSTITUTED SEDIMENT
- ABIOTIC FACTORS
- REFERENCE TOXICANTS
- SPECIES AND STRAIN SENSITIVITY

RESEARCH NEEDS FOR STANDARDIZATION OF HYALELLA AZTECA (cont).

- INTER-LABORATORY COMPARISONS
- LIFE HISTORY AND CHRONIC INDICATORS OF TOXICITY
- SPIKING METHODS AND POSITIVE CONTROLS
- DILUTION STUDIES AND MIXTURES
- LABORATORY TO IN SITU COMPARISONS

Discussion of Desirable and Necessary Attributes for Marine and Estuarine Sediment Toxicity Tests, and the Use of Ampelisca abdita, Rhepoxynius abronius, Leptocheirus plumulosus, and Eohaustorius estuarius in Marine and Estuarine Sediments

Richard C. Swartz, U.S. EPA Environmental Research Laboratory - Pacific Division

I. Description of Acute Amphipod Sediment Toxicity Test

II. Research and Regulatory Applications

III. Limitations and Advantages

IV. Necessary and Desirable Attributes of Acute Sediment Toxicity Tests

A. Species selection
   1. Relative sensitivity (field and toxicological data)
      a. Other species
      b. Size
      c. Sex
   2. Ecological importance/relevance
   3. Economic importance
   4. Habitat
   5. Substrate relation
      a. Pelagic
      b. Epibenthic
      c. Infaunal - tube dwelling
      d. Infaunal - free burrowing
   6. Availability
      a. Field collection
      b. Culture method
   7. Laboratory compatibility
   8. Zoogeography
   9. Compatibility with bioaccumulation/chronic tests

B. Method development and standardization
   1. Written standard method
   2. Sediment toxicity database
      a. Field sediment
      b. Spiked sediment
   3. Control responses
      a. QA/QC - collection/culture sediment
      b. QA/QC - reference toxicant
      c. Experimental - negative
      d. Field - reference sediment
   4. Statistical power
   5. Tolerance limits of species/method
      a. Sediment grain size
      b. Temperature
      c. Salinity
      d. Sediment organic enrichment
      e. Ammonia
      f. Seasonality
   6. Sediment collection/processing/storage
   7. Field validation
   8. Interlaboratory comparison

V. Amphipod Species

A. Rhepoxynius abronius
B. Ampelisca abdita
C. Eohaustorius estuarius
D. Leptocheirus plumulosus

VI. Chronic Test Methods

VII. Other Species

TABLE 1—*Precision of the benthic bioassay in relation to sample size and replication.*[a]

Number of Amphipods per Replicate					
10			20		
No. of Replicates	δ	δ/c	No. of Replicates	δ	δ/c
2	6.80	71.6			
4	2.66	28.0	2	8.55	45.0
6	1.94	20.4	3	4.44	23.4
8	1.60	16.8	4	3.35	17.6
10	1.38	14.5	5	2.80	14.7
12	1.25	13.2	6	2.45	12.9
14	1.14	12.0	7	2.20	11.6
16	1.05	11.0	8	2.02	10.6
18	0.98	10.3	9	1.89	10.0
20	0.93	9.8	10	1.76	9.3

[a] δ is the difference between two survival means for which the bioassay is 75% certain of detecting statistical significance ($P < 0.05$) [*16*]. δ/c expresses the precision estimate as a percent of the normal control survival ($c = 19.0$ for $n = 20$; $c = 9.5$ for $n = 10$).

Field Validation

Parameter	Toxic Sites	Non-Toxic Sites
Benthic Biomass	9.4	50.3
Benthic Density	450	2000
Species Richness	21	70
Amphipod Density	0.8	33
Rhepoxynius density	0	23
Eh	-54	+223
BOD	13	4
Oil/Grease	15	4
Cadmium	29	11
Chromium	670	370
DEHP	16	4
DDE	5	3

1. Written Protocol

Biology of Test Species
- all species selection factors

Limitations of Method
- environmental factors (grain size, salinity, etc.)
- variability/statistical power
- field relevance

Logistics
- exposure chamber
- duration
- sequence of events
- quarantine

	Rhepoxynius	Ampelisca	Eohaustorius	Leptocheirus
Sensitivity	High	High	High	High
Ecological Importance	High	High	High	High
Economic Importance	Low	Low	Low	Low
Habitat	Marine	Marine - Mid Estuarine	Low - Mid Estuarine	Mid - High Estuarine

	Rhepoxynius	Ampelisca	Eohaustorius	Leptocheirus
Substrate Relation	Free burrowing	Tube dwelling	Free burrowing	Tube dwelling
Availability	Field	Field, Culture	Field	Field, Culture
Lab Compatibility	High	High	High	High
Zoogeography	Pacific	Atlantic, Gulf San F. Bay	Pacific	Atlantic

	Rhepoxynius	Ampelisca	Eohaustorius	Leptocheirus
Chronic Test	No	Yes	No	Yes
Bioaccumulation Test	Low	Low	Low	Low
Protocol	ASTM	ASTM	ASTM	ASTM
Tox. Data Base	Extensive	Extensive	Moderate	Limited

	Rhepoxynius	Ampelisca	Eohaustorius	Leptocheirus
Controls/QAQC	++++	+++	+++	+
Statistical Power	15-25% change from reference with 5 replicates			
Field Validation	+++	++	+	+
Interlab Comparison	+++	++	-	+

	Rhepoxynius	Ampelisca	Eohaustorius	Leptocheirus
Knowledge of Tolerance Limits	+++	++	+	+
Tolerance Limits				
Salinity	> 25 ppt	Broad	Broad	Broad
Grain Size	Clays	Sands?	Clays?	Sands?
Ammonia	?	?	?	?
Total Organic C	?	?	?	?
Seasonality	< 2X	?	?	?

Other Species

Grandidierella japonica
Lepidactylus dytiscus
Corophium spp
Neanthes arenaceodentata
Meiofauna

Other Tests

Microtox
Bivalve Larval Survival/Growth/Development
Echinoderm Larval Survival/Growth/Development
Benthic Recolonization

Issues and Research Needs

Written Protocol - ASTM/EPA format
Culture Protocol: Leptocheirus/Ampelisca
Sensitivity of Cultured vs Field-collected Amphipods
* Shipping/Handling/Acclimation
Nutrition
Comparative Toxicology
* Reference Toxicant Control
* Reference Sediment QA/QC
Statistical Power: Compare variability among species
Tolerance Limits
* Ammonia
* Grain Size, except Rhepoxynius
Organic Enrichment
Salinity, except Rhepoxynius
Seasonality
Light: Intensity/photoperiod
* Field Validation
* Interlaboratory Comparison
* Sediment Collection, Processing, Storage

Chronic Test Methods

Test Species

Leptocheirus plumulosus, *Ampelisca abdita*

Response Criteria

Mortality, Growth, Reproduction, Population Dynamics

Key Issues

Nutrition

Narrow Tolerance Limits

Chronic Control QA/QC

Relative Sensitivity of Acute and Chronic Tests

Bioaccumulation of Sediment-Associated Contaminants: Significance, Current Status, and Future

Peter Landrum, NOAA Great Lakes Environmental Research Laboratory - Ann Arbor, MI

I. Significance of Bioaccumulation

- A. Role in toxicity assessment
  - 1. Aquatic species
  - 2. Human health

II. Picture of the Problem

- A. Complexity of the exposure environment
- B. Current mechanistic model

III. Criteria for Bioaccumulation Organisms

- A. Examples of organisms

IV. Factors Affecting Bioaccumulation

- A. External factors
- B. Physiological factors
  - 1. Behavior
    - a. Importance of feeding
    - b. Feeding mechanism
    - c. Feeding selectivity

V. Status

- A. Field data

VI. Future - Kinetics

- A. Models
- B. Field validation

BIOACCUMULATION

The accumulation of contaminants from all sources, food and water.

BIOACCUMULATION FACTOR

The ratio of the steady state concentration in the organism resulting from multiple source accumulation to one of the source concentrations

WHAT IS THE SIGNIFICANCE OF BIOACCUMULATION?

1. Toxicity is based on exposure - Bioaccumulation provides a measure of exposure

2. Food-chain transfer depends on bioaccumulation

TISSUE RESIDUE FOR TOXICITY ASSESSMEI

Acute Narcosis (50% mortality) 2 - 6 mmol kg^{-1}

Neutral Narcotics seem to act as additive toxins

Specific mechanisms of action (acute mortality) < 0.5 mmol kg^{-1}

e.g. Lindane and dieldrin 6.3 µmol kg^{-1}

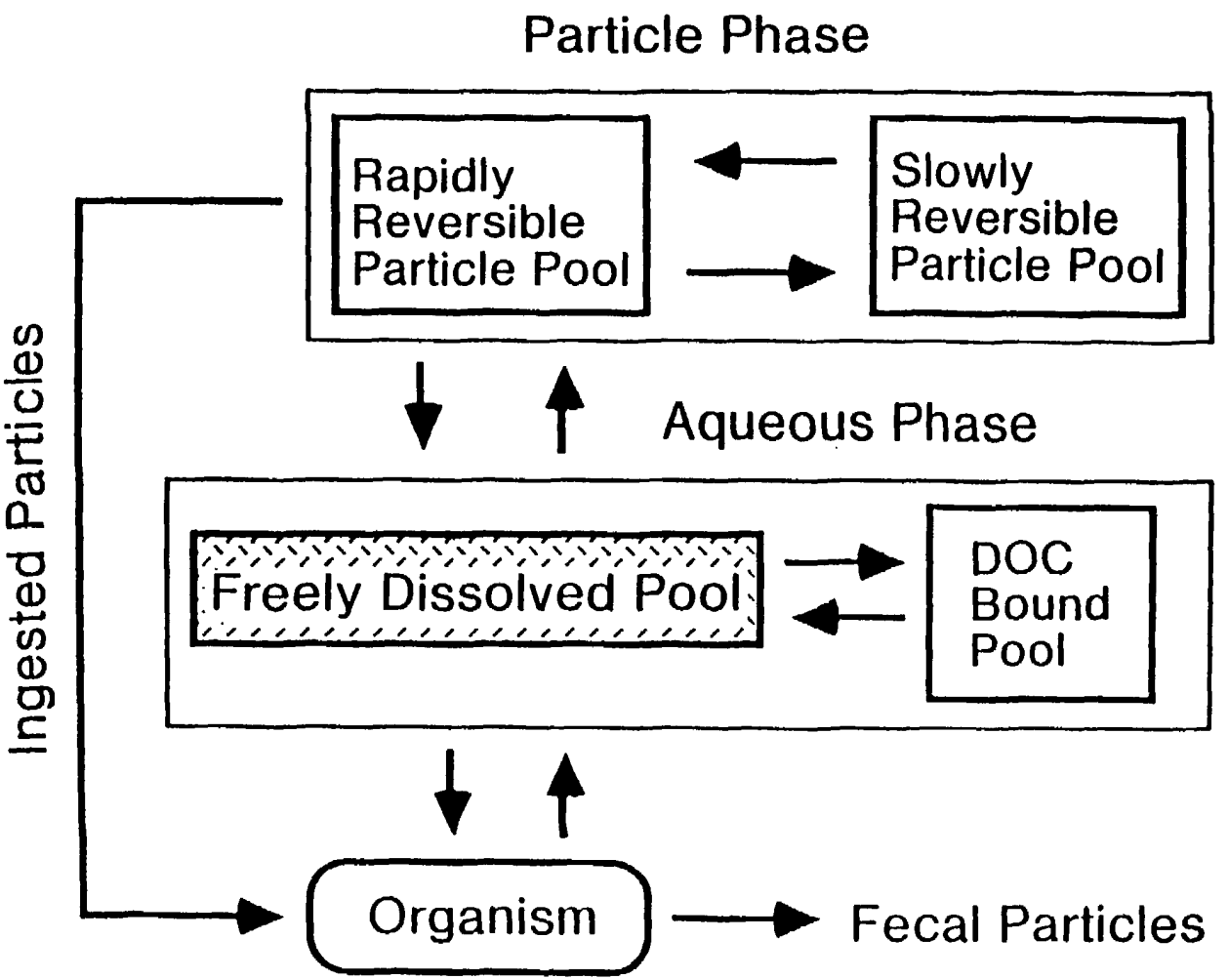
Chronic Narcosis (50% mortality) 0.2 - 0.6 mmol kg^{-1}

Scope for growth 4 µmol kg^{-1}

bromophos and fenthion act as narcotics in guppy requiring 5 to 12 mmol kg^{-1} for acute mortality

Chlorthion (0.41 mmol kg^{-1}) and methidathion (2.5 µmol kg^{-1}) act as cholinesterase inhibitors in the guppy

pentachlorophenol acts as a narcotic in M. relicta (4.5 mmol kg^{-1}) and apparently as a respiratory inhibitor in Diporeia spp. (0.58 - 0.91 mmol kg^{-1})


Particle Phase
Rapidly Reversible Particle Pool
Slowly Reversible Particle Pool
Aqueous Phase
Freely Dissolved Pool
DOC Bound Pool
Ingested Particles
Organism
Fecal Particles

CRITERIA FOR BIOACCUMULATION ORGANISMS

Size - preferably large enough to be easily handled and supply significant biomass

Class - infaunal benthos, organism utilizes sediment detritus for food supply

Culture - easily cultured or field collected

Tolerance - organisms should be tolerant of variation in sediment composition and contaminant concentrations

Lipid content - high lipid content is preferable for accumulation of neutral organic contaminants

Minimal biotransformation capability

EXAMPLES OF FRESHWATER BIOACCUMULATION ORGANISMS

Diporeia spp. - abundant, require low temperature, high lipid content (25 - 50% dry weight), tolerates wide range of sediment composition, size 6 mg wet weight, infaunal benthos, must be field collected, tolerate high salinity (20 $^{o}/_{oo}$), moderate data base available for accumulation, kinetics and toxicity, very selective feeders

Lumbriculus variegatus - easily cultured, infaunal benthos, can work at room temperature, tolerates wide range of sediment composition, wet weight 5 mg, tolerant of high chemical concentrations

Hexagenia limbata - can be cultured with difficulty, readily field collected, tube dweller but ingests sediment under laboratory conditions, lipid content 3.5 - 15% dry weight, tolerate a wide range of temperature and can be used at room temperature, sensitive to contaminants and sediment composition

FACTORS AFFECTING BIOACCUMULATION

Environmental Factors

- Contaminant Properties
- Sediment Characteristics
- Environmental Conditions e.g. temperature, sunlight

Physiological Factors

- Biotransformation
- Behavior e.g. feeding behavior
- Growth
- Reproductive State
- Health

BEHAVIOR CHANGES AFFECTING BIOACCUMULATION

Feeding - feeding uncontaminated food can result in lower exposure as organism obtains significant amounts of sediment-associated contaminants from ingestion

Food Supply - depletion of food supply in the sediment can result in decreased exposure over time

Feeding Behavior - toxicity can alter the feeding response of organisms reducing their accumulation

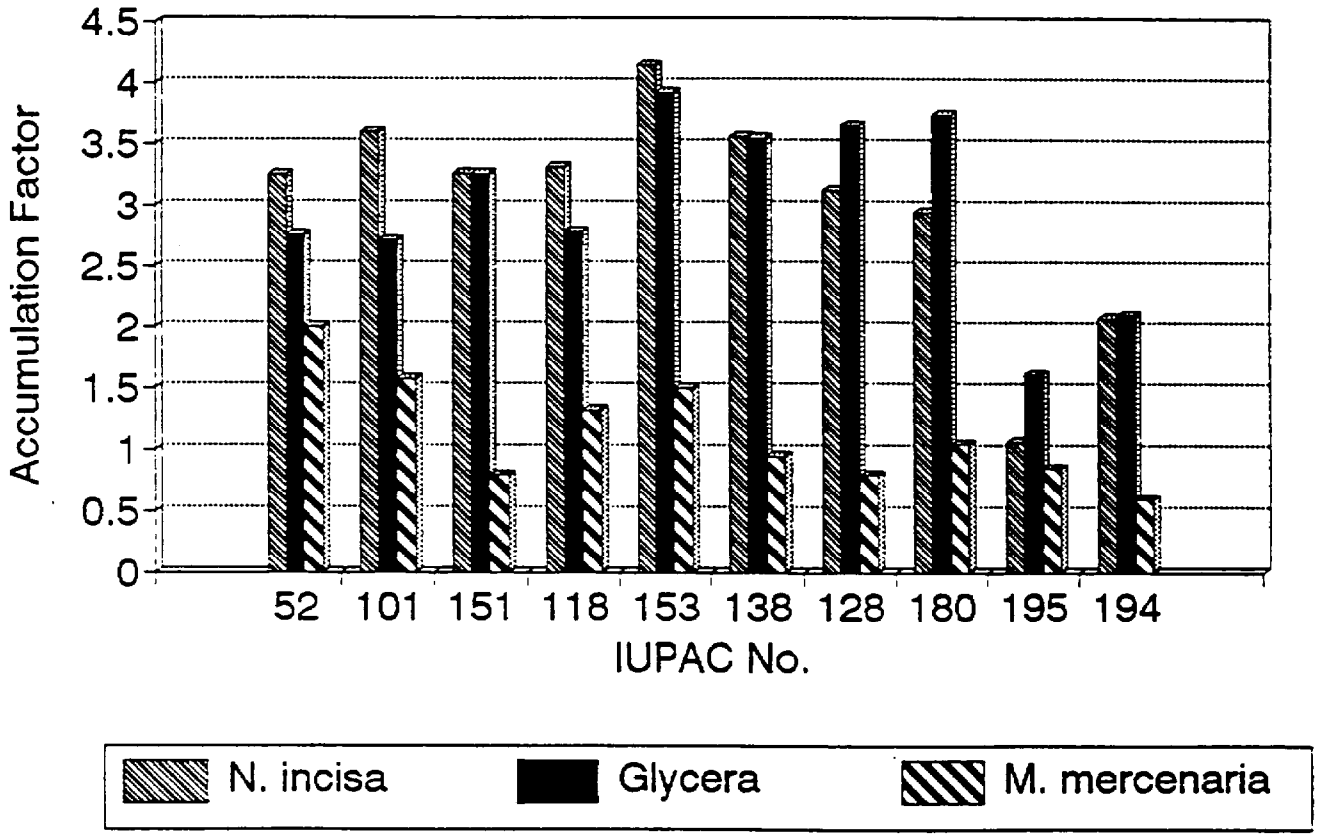
Sediment Avoidance - avoiding sediment contact reduces exposure to both contaminated food and interstitial water

Feeding Selectivity - The organism should be as non selective as possible

IMPORTANCE OF FEEDING

Duration	Feeding (DPM/g)	Not Feeding (DPM/g)
24 h	5,968 ± 2,238	5,265 ± 1,794
72 h	12,989 ± 3,289	9,836 ± 2,921
120 h	21,128 ± 3,608	13,261 ± 2,188
168 h	29,221 ± 6,947	13,113 ± 2,420

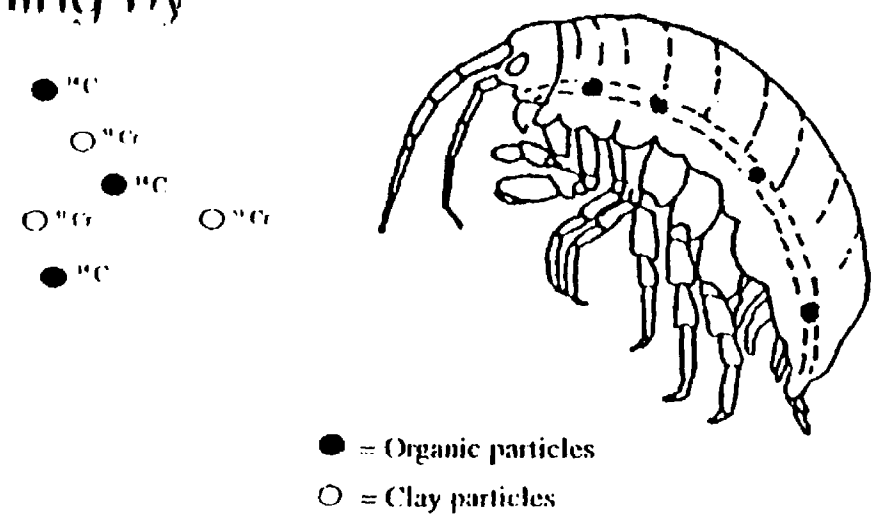
Accumulation Factors
For PCB Congeners



Lake et.al 1990

Program Accomplishments (Cont.)

- Examined two experimental methods for measuring the assimilation efficiency of ingested contaminants.
  - Dual-label tracer approach could not account for selective feeding by *Diporeia*.



DOES CURRENT METHODOLOGY PROVIDE NEEDED INFORMATION?

1. Current methods are point estimates
2. Data is conditional on experimental conditions
3. Changes in organism physiology will change results
4. Current methods can only account for a single source

WHY KINETICS?

1. Predict accumulation at other than steady-state

2. Predict steady-state when organism requires long (> 1 month) to achieve steady-state

3. Examine mechanisms affecting accumulation and loss of contaminants

4. Predict accumulation from multiple routes of exposure

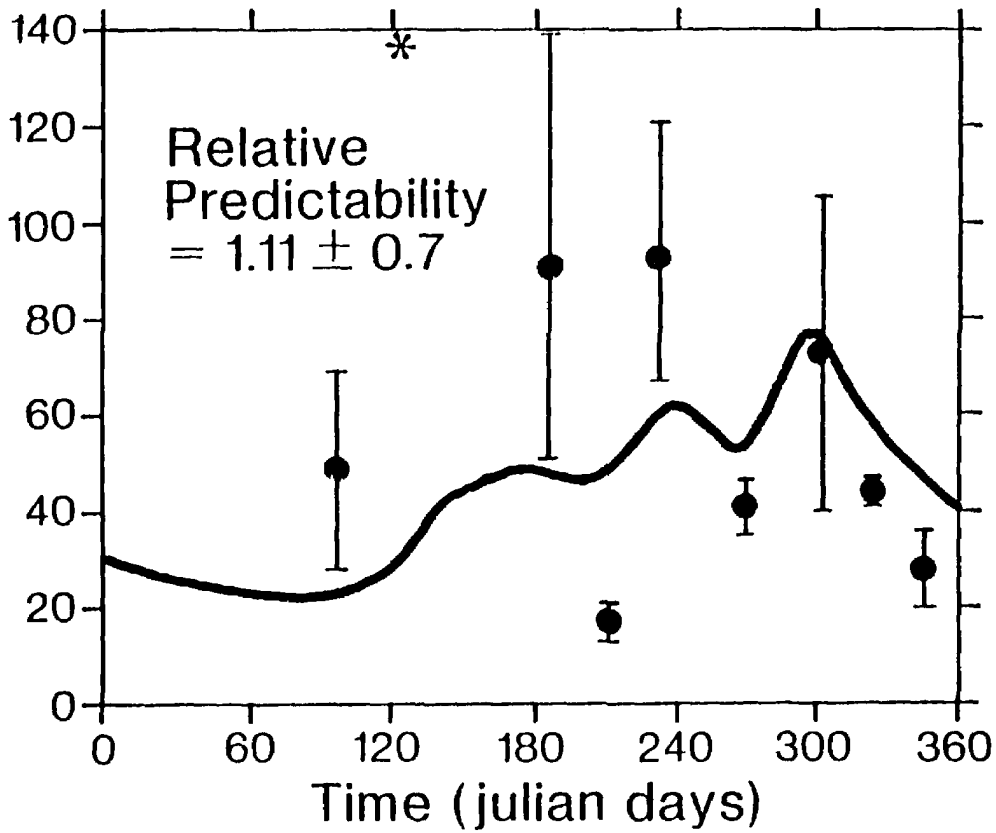
5. Account for changes in physiology and environmental conditions e.g. growth, reproductive state, temperature

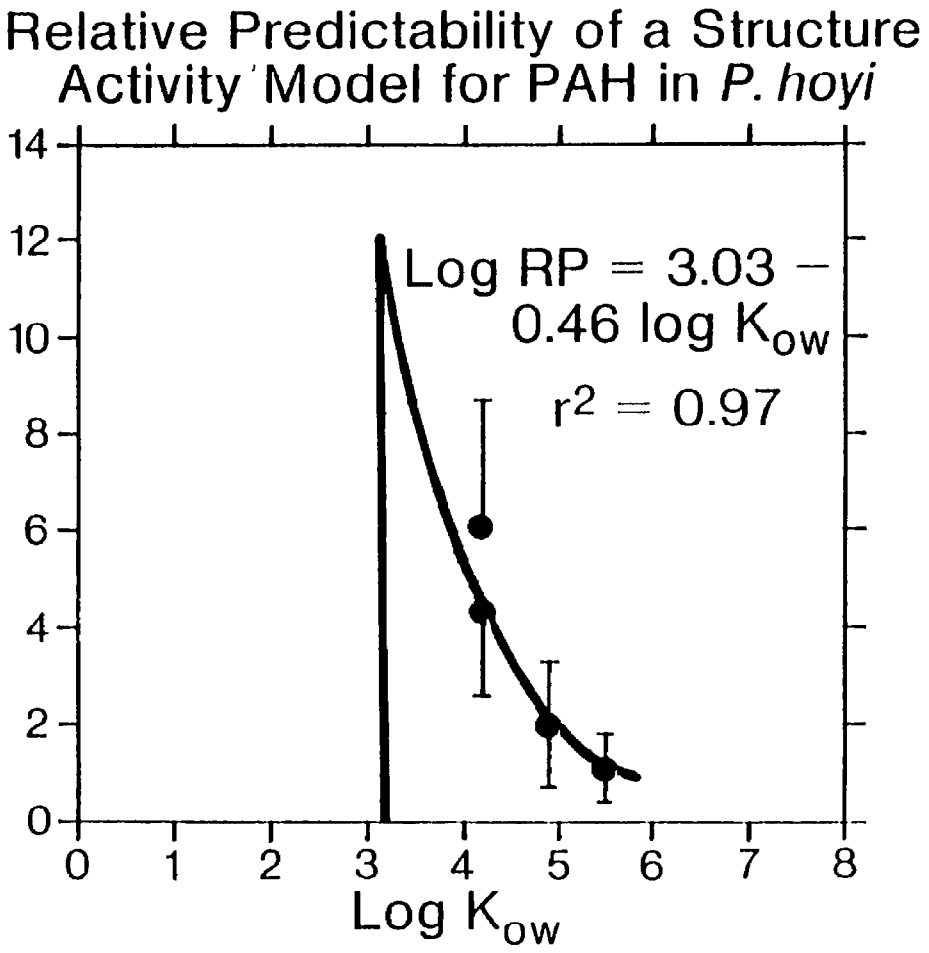
6. Predict effects using the tissue residue approach

MODELS

- Compartment Based
  - Rate Coefficient
  - Fugacity
  - Clearance Volume
- Physiologically Based
  - Physiologically Based Pharmacokinetic Models
  - Bioenergetics Based

Concentration of BaP (ng/g wet weight) in *P. hoyi* from Structure Activity Model at Reduced Water Concentration




Relative Predictability of a Structure Activity Model for PAH in *P. hoyi*
Log RP = 3.03 − 0.46 log K_{ow}
r^2 = 0.97
14
12
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8
Log K_{ow}

Bioaccumulation of Sediment-Associated Contaminants: Present Status, Laboratory Methods, and Related Research Needs

Henry Lee II, U.S. EPA Environmental Research Laboratory - Pacific Division

I. Important of Bioaccumulation of Sediment-Associated Compounds

II. Methods to Measure/Predict Bioaccumulation of Sediment-Associated Compounds

- A. Criteria to choose among the methods

III. Equilibrium Participating Bioaccumulation Model

- A. Use as screening tool
- B. Limitations/uncertainties with equilibrium predictions
- C. Lab/kinetic alternatives to partitioning paradigm

IV. 28-Day Bedded Sediment Bioaccumulation Test

- A. Status of "standard 28-day" sediment bioaccumulation test
- B. Test duration
  1. Why need a 28-day test vs. 10-day test
  2. Adequacy of 28-day tests for slowly accumulated compounds
- C. Organism selection
  1. Selection criteria
  2. Why need sediment ingesting organism
  3. Recommended bioaccumulation species
- D. Laboratory methods
  1. No feeding of test organisms
- E. Experimental design
  1. Number of replicates and statistical power
  2. Pseudoreplication
- F. How proposed methods differ from those in the "green book"

V. Toxicokinetic Bioaccumulation Models/Tests

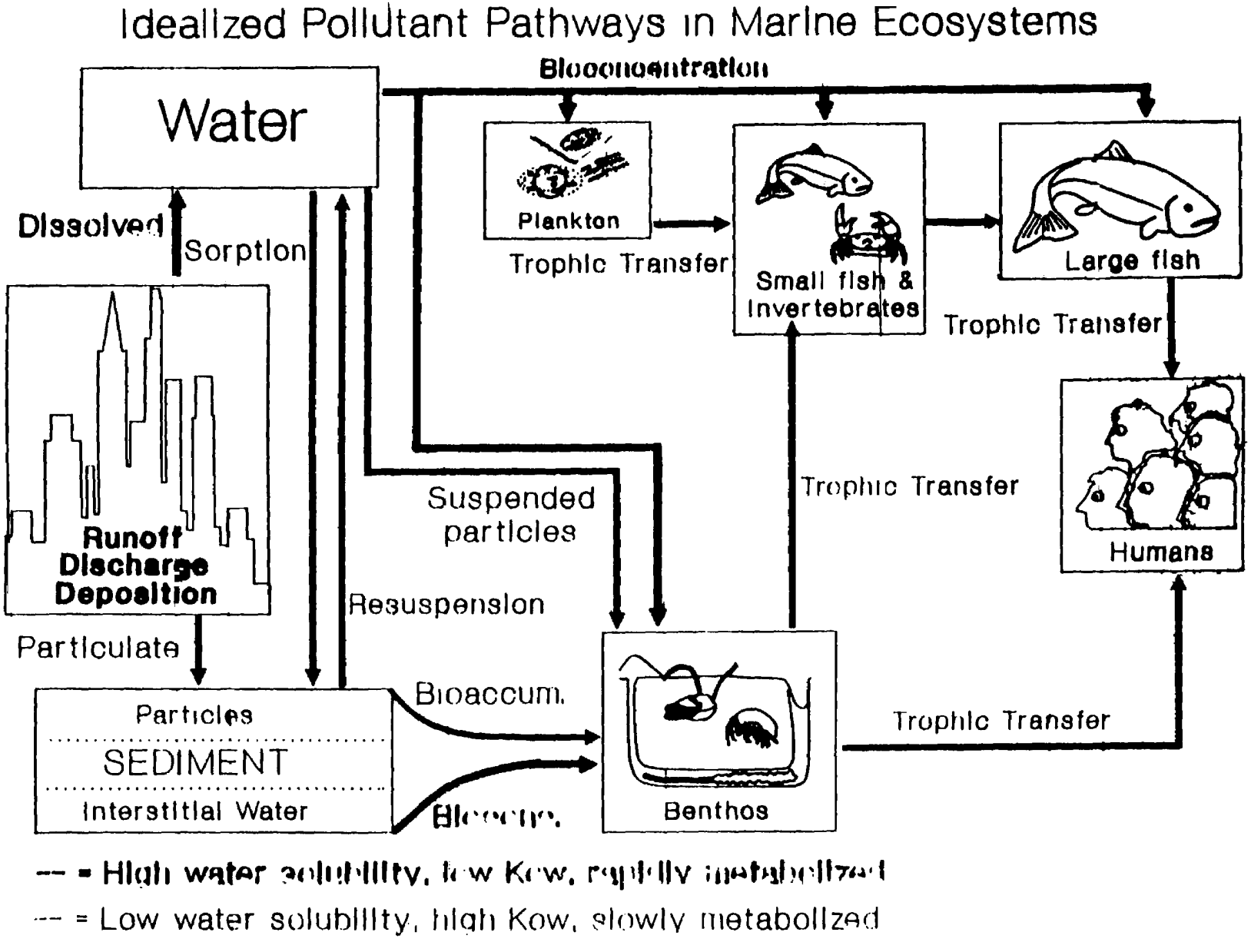
- A. Equilibrium vs. non-equilibrium exposures
- B. Modifications of laboratory procedures

VI. Evaluation of Bioaccumulation Results

A. Criteria for "reference" sites

VII. Research Needs

A. Related to 28-day bioaccumulation test
B. Field validation
C. Round robin
D. Lipid methods
E. Research needs related to other methods of predicting bioaccumulation
F. Resuspended sediment tests


Idealized Pollutant Pathways in Marine Ecosystems
Water
Bioconcentration
Dissolved
Sorption
Plankton
Trophic Transfer
Small fish & Invertebrates
Large fish
Trophic Transfer
Runoff
Discharge
Deposition
Suspended particles
Trophic Transfer
Humans
Resuspension
Particulate
Bioaccum.
Particles
SEDIMENT
Interstitial Water
Benthos
Trophic Transfer
-- = High water solubility, low Kow, rapidly metabolized
-- = Low water solubility, high Kow, slowly metabolized

PREDICTING BIOACCUMULATION OF SEDIMENT - ASSOCIATED POLLUTANTS BY INFAUNAL ORGANISMS

- **Field Approach:**
  measure tissue residues in species collected at surrogate site

- **Bioaccumulation Test:**
  measure tissue residues in species exposed to sediment collected at a surrogate site

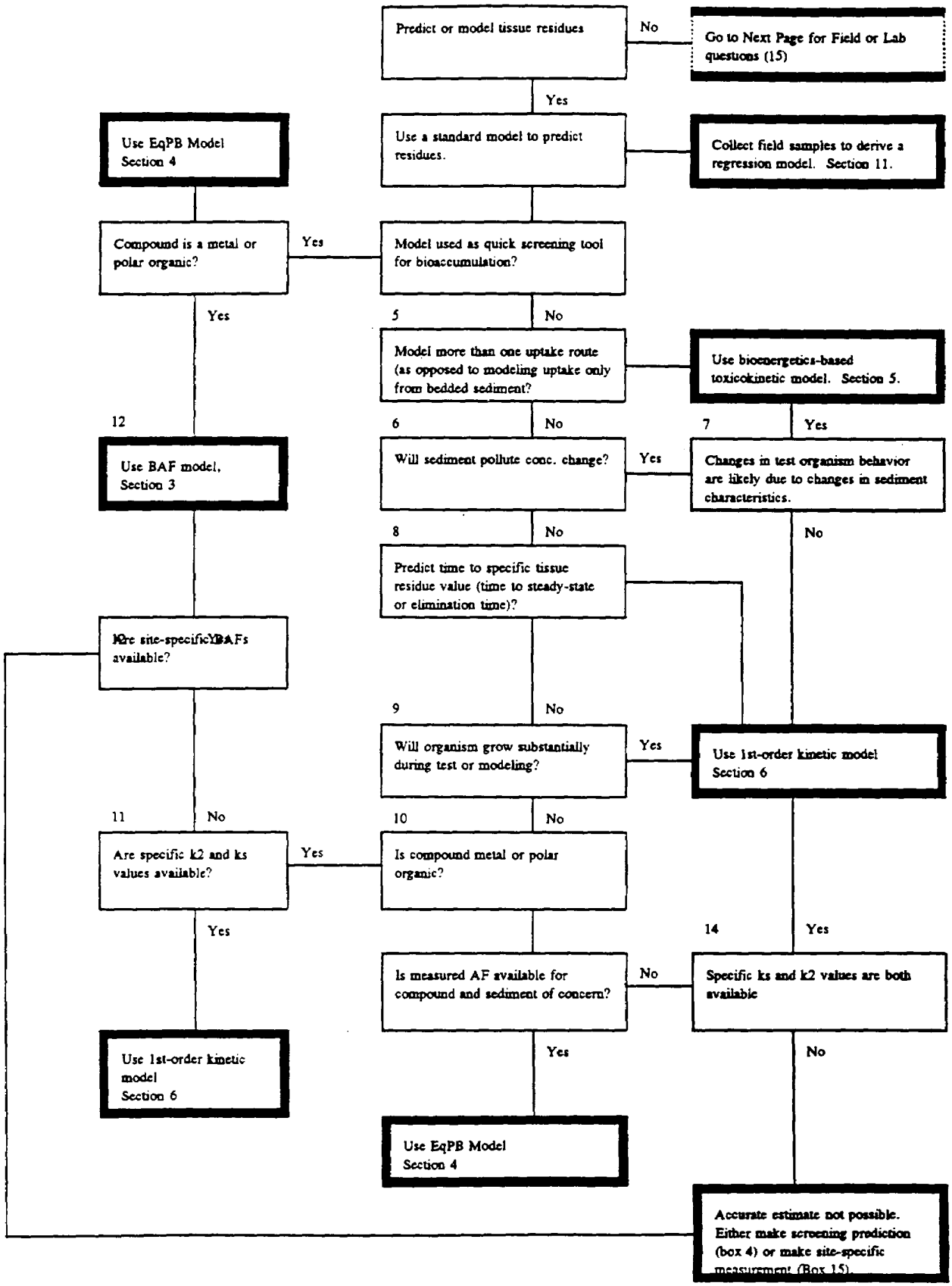
- **Bioaccumulation Factors (BAF):**
  ratio of concentrations in tissue to sediment

- **Thermodynamic Partitioning Model:**
  based on chemical partitioning normalizing for lipids and TOC but only for neutral organics

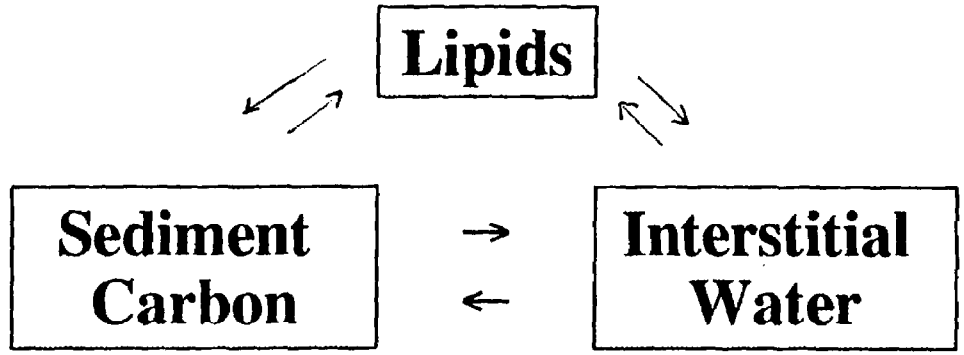
- **1st Order Kinetic Model:**
  tissue residues modeled as balance between rate of uptake and rate of loss

- **Toxicokinetic Model:**
  tissue residues modeled as function of bioenergetics of the organism

Figure 2.1: Summary of questionnaire choices. Numbers above decision boxes refer to questionnaire choices.



EQUILIBRIUM PARTITIONING BIOACCUMULATION MODEL



$$Ctss/L = (Cs/TOC)^* AF$$

or

$$AF = (Ctss/L/(CS/TOC)$$

Where:

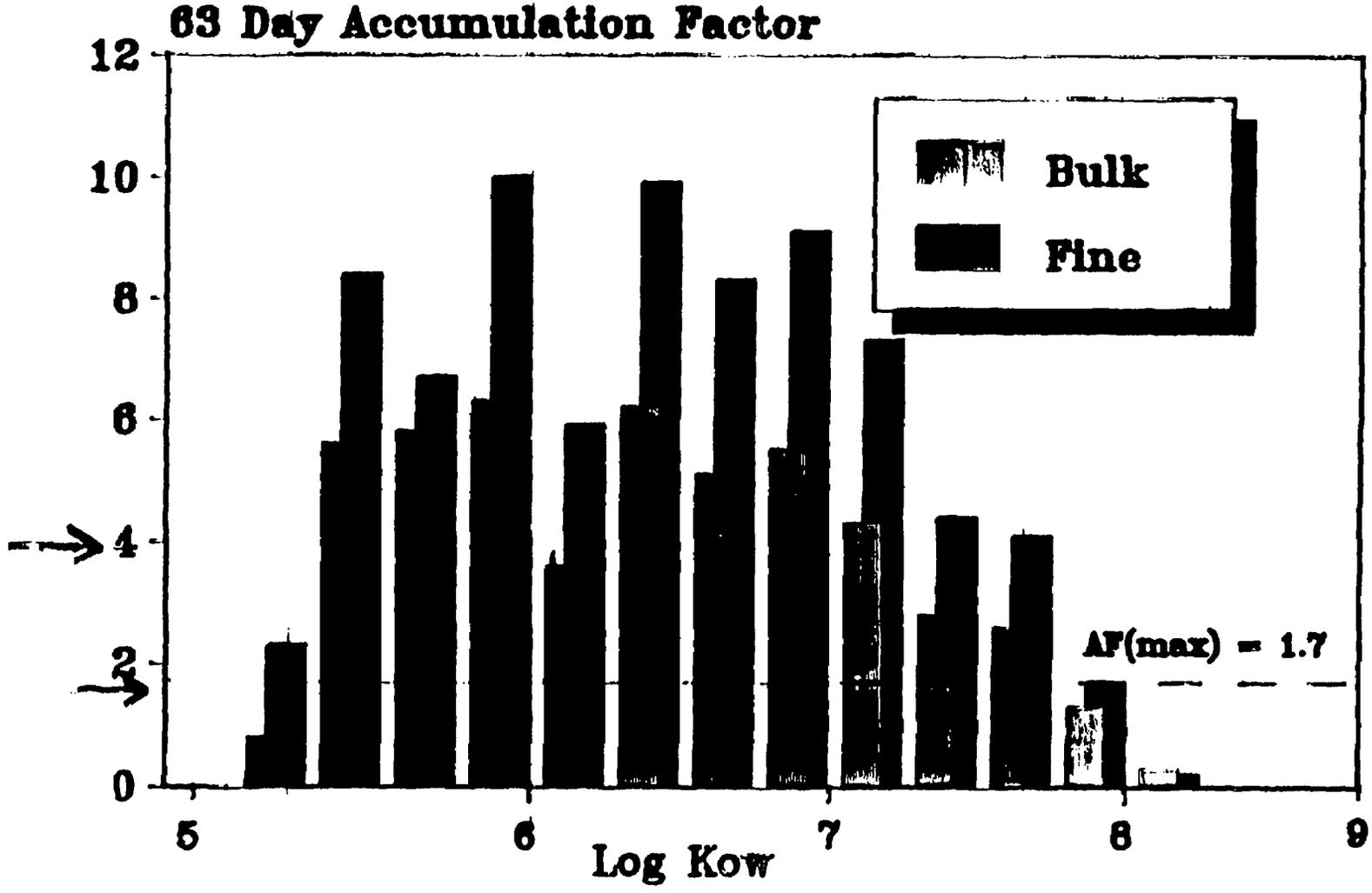
Ctss = Tissue conc. at steady-state (ug/g)
L = Lipid content (g/g)
TOC = Total organic carbon in sediment (g/g)
Cs = Sediment conc. (ug/g)
AF = Accumulation Factor (g carbon/g lipid)

1) Tissue residues cannot exceed the concentration set by partitioning (AF < =2)

2) AFs do not vary among species, sediments, or compounds.

COMPOUND	ORGANISM	AF	TOC%	LIPID%	REFERENCE
HCBP	*Yoldia limatula*	1.7	1.9		McElroy and Means, 1988
HCBP	*Yoldia limatula*	0.9	4.0		McElroy and Means, 1988
HCBP	*Yoldia limatula*	8.2[6]			Lake et al., 1990
HCBP	*Yoldia limatula*	5.1[6]			Lake et al., 1990
HCBP	*Nephtys incisa*	0.4	1.9		McElroy and Means, 1988
HCBP	*Nephtys incisa*	0.2	4.0		McElroy and Means, 1988
HCBP	*Nephtys incisa*	16.6[7]			Lake et al., 1990
HCBP	*Nephtys incisa*	7.1[8]			Lake et al., 1990
HCBP	*Nephtys incisa*	7.1[7]			Lake et al., 1990
HCBP	*Nephtys incisa*	3.8[8]			Lake et al., 1990
HCBP	*Glycera sp.*	3.9			Lake et al., 1990
HCBP	*Mercenaria mercenaria*	1.5			Lake et al., 1990
HCBP	*Nereis virens*	4.8	1.1		Brannon et al., (manuscript)
HCBP	*Nereis virens*	1.4			Brannon et al., (manuscript)
HCBP	*Nereis virens*	4.8			Brannon et al., (manuscript)
HCBP	*Nereis virens*	1.4	5.7	7.6	Pruell et al., (manuscript)
HCBP	*Macoma nasuta*	4.8	1.1		Pruell et al., (manuscript)
HCBP	*Macoma nasuta*	0.5			Pruell et al., (manuscript)
HCBP	*Macoma nasuta*	1.6	0.8	4.6	Boese et al., (manuscript)
HCBP	*Macoma nasuta*	2.7	1.3	4.6	Boese et al., (manuscript)
HCBP	*Macoma nasuta*	4.0	2.5	4.6	Boese et al., (manuscript)
HCBP	*Macoma nasuta*	1.8	5.7	1.3	Pruell et al., (manuscript)
HCBP	*Diporeia sp.*	0.7[9]	0.7		Landrum (pers. comm.)
HCBP	*Oligochaetes*	1.2[10]	3.6	6.6	Olivier, 1984
HCBP	*Palaemonetes pugio*	2.1	5.7	1.6	Pruell et al., (manuscript)
	Bivalve Mean	4.4			
	Polychaete Mean	4.7			
	Overall Mean	4.4			
PESTICIDES					
a-chlor	*Yoldia limatula*	4.0			Lake et al., 1987
a-chlor	*Nephtys incisa*	4.2			Lake et al., 1987
g-chlor	*Yoldia limatula*	4.5			Lake et al., 1987
g-chlor	*Nephtys incisa*	5.9			Lake et al., 1987
	Overall Mean	4.7			
DDD	*Macoma nasuta*	1.0	0.9	5.5	Ferraro et al., 1990
DDD	*Macoma nasuta*	0.4	0.8	5.5	Ferraro et al., 1990
DDD	*Macoma nasuta*	0.7	3.7	5.5	Ferraro et al., 1990
DDD	*Macoma nasuta*	0.7	4.0	5.5	Ferraro et al., 1990
DDD	*Macoma nasuta*	0.5	5.1	5.5	Ferraro et al., 1990
DDD	*Macoma nasuta*	0.5	7.4	5.5	Ferraro et al., 1990
DDD	*Yoldia limatula*	4.0			Lake et al., 1987
DDD	*Yoldia limatula*	4.2			Lake et al., 1987
DDD	*Nephtys incisa*	4.2			Lake et al., 1987
DDD	*Nephtys incisa*	4.8			Lake et al., 1987
	Bivalve Mean	1.5			
	Polychaete Mean	4.5			
	Overall Mean	2.1			
DDE	*Macoma nasuta*	2.8	0.9	5.5	Ferraro et al., 1990
DDE	*Macoma nasuta*	0.7	0.8	5.5	Ferraro et al., 1990
DDE	*Macoma nasuta*	1.3	3.7	5.5	Ferraro et al., 1990
DDE	*Macoma nasuta*	1.1	4.0	5.5	Ferraro et al., 1990
DDE	*Macoma nasuta*	0.7	5.1	5.5	Ferraro et al., 1990
DDE	*Macoma nasuta*	1.1	7.4	5.5	Ferraro et al., 1990
DDE	Oligochaetes	0.9[10]	3.6	6.6	Olivier, 1984
DDE	Oligochaetes	1.0[10]	4.6	6.6	Olivier, 1984
DDE	Oligochaetes	1.3[10]	6.0	6.6	Olivier, 1984
	Bivalve Mean	1.3			
	Overall Mean	1.2			

PCB Congener AFs in Fine and Bulk Sediments



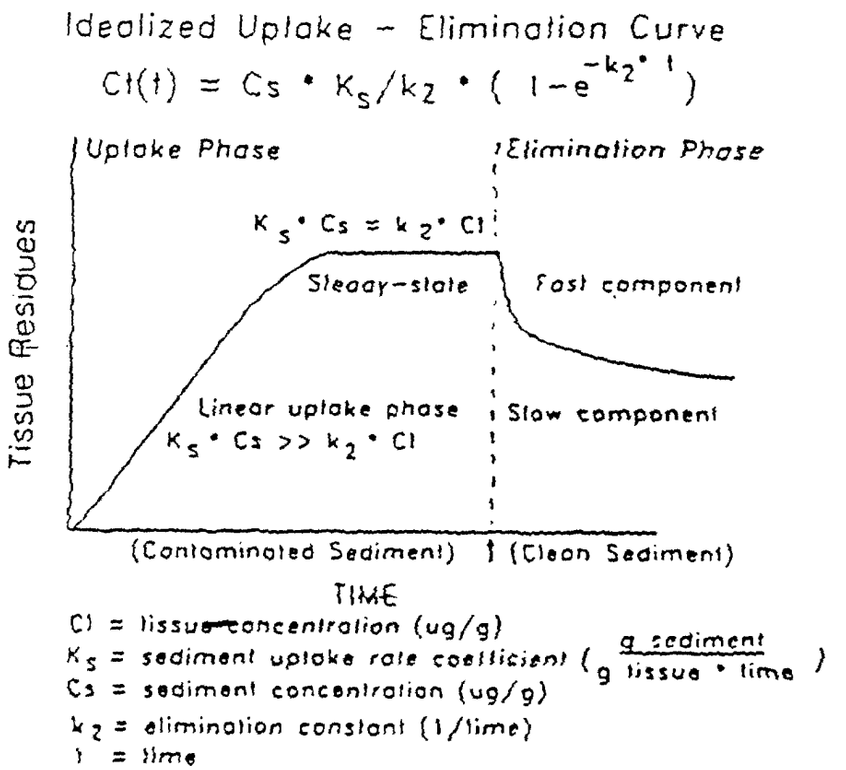
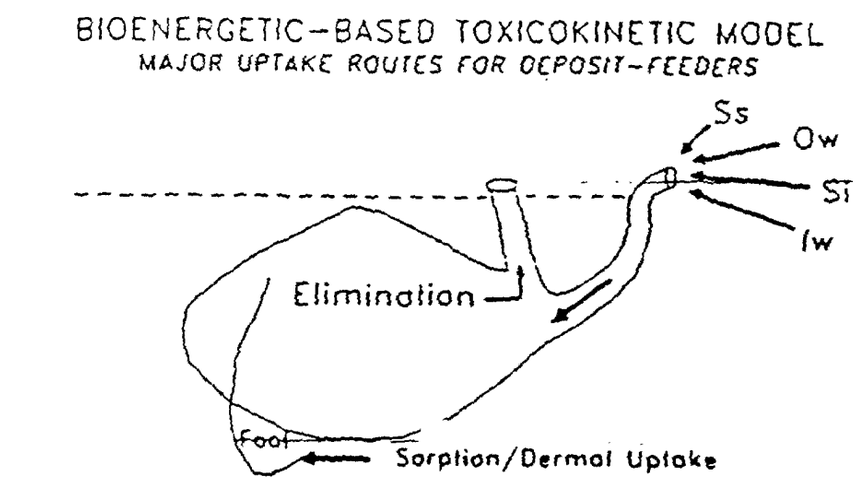
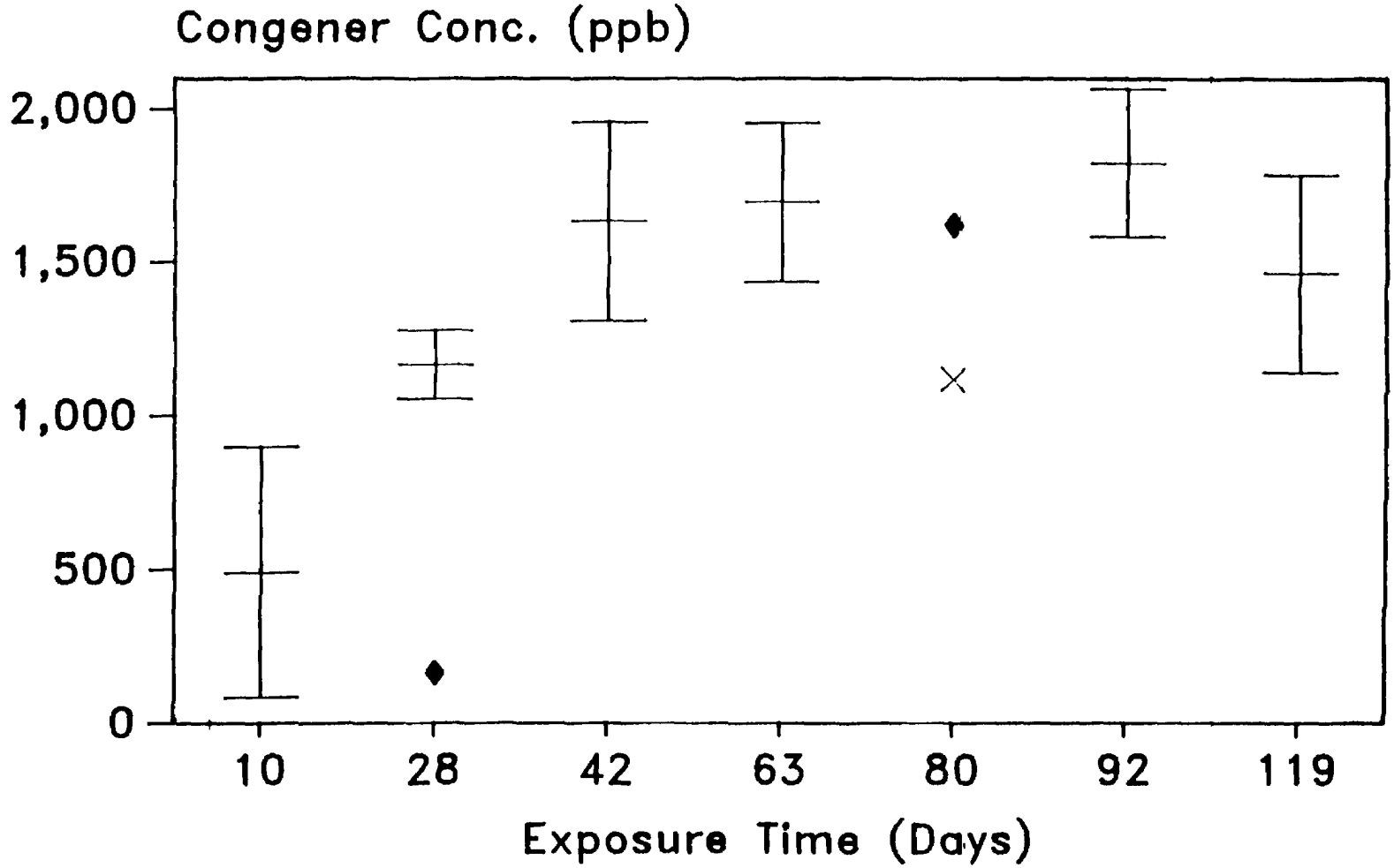


Figure 2. Idealized uptake — elimination curve.

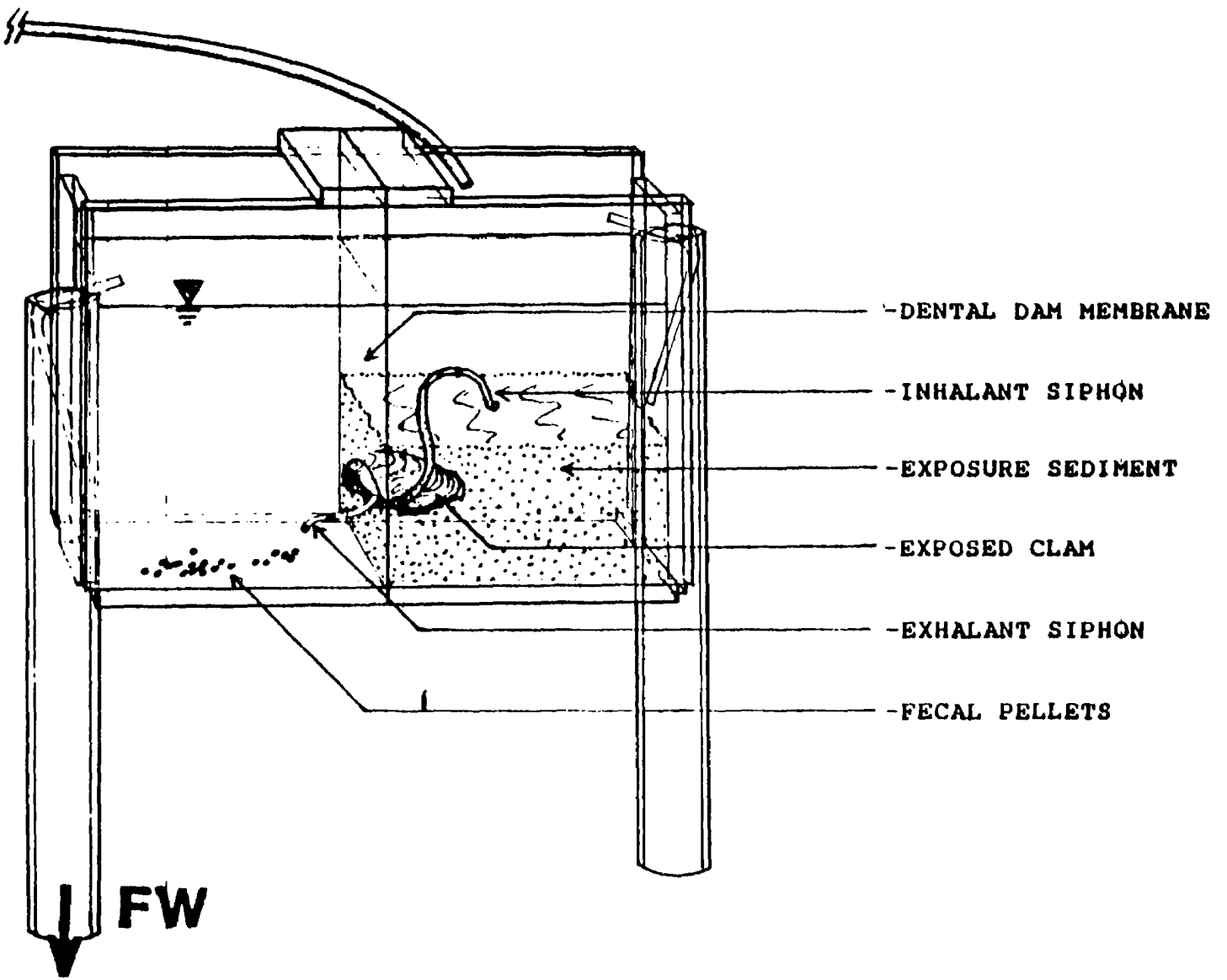


Predicted vs Observed Tissue Residues
PCB 105 in Macoma Nasuta



I TR (95% CI) × AF 4 ♦ AF Empirical AF 1.72 — Kinetic Model

CLAMBOX



EVALUATION OF DREDGE MATERIAL BEDDED - SEDIMENT BIOACCUMULATION TEST

- **OBJECTIVE:** Measure tissue residues in infaunal organisms resulting from exposure to dredge material

- **PREVIOUS APPROACH:** 10-day bedded-sediment bioaccumulation test to estimate "bioaccumulation potential"

- **PRESENT APPROACH:** 28-day bedded-sediment bioaccumulation test to estimate "steady-state" tissue residues (10 day - test if only metals present). Used in Tier III

- **STATUS:** EPA Guidance Document produced and referenced in Implementation Manual. Guidance document in ASTM review

WHY CONDUCT SEDIMENT BIOACCUMULATION TESTS?

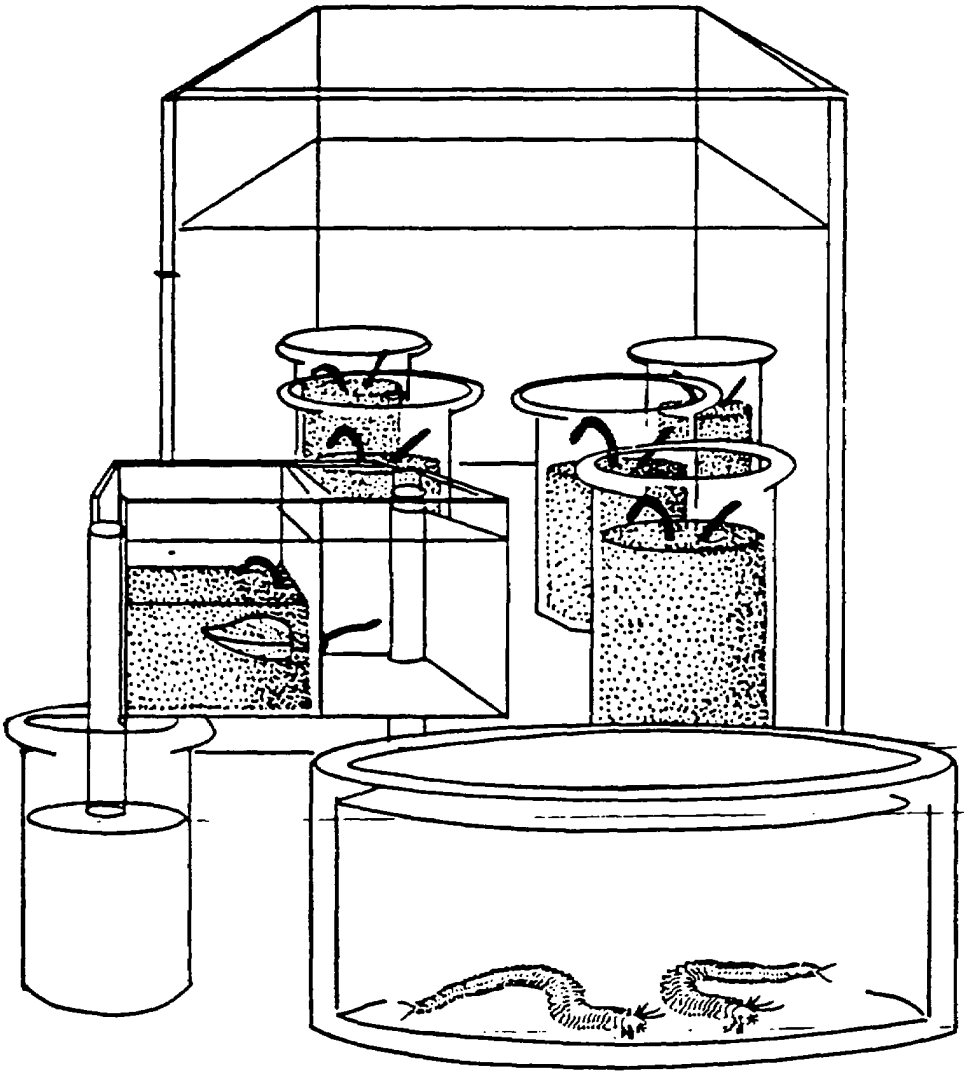
- HAZARD IDENTIFICATION (BIOACCUMULATION POTENTIAL)
- ASSESS DREDGE MATERIALS
- ASSESS EXPOSURE TO BENTHIC ORGANISMS FOR ECOLOGICAL RISK ASSESSMENTS
- ASSESS SEDIMENTS FOR HUMAN HEALTH EFFECTS
- ASSESS EXPOSURE TO DEMERSAL FISHES, MARINE MAMMALS, AND BIRDS
- TEST OR DERIVE SEDIMENT QUALITY CRITERIA
- SCIENTIFIC UNDERSTANDING OF SEDIMENT BIOAVAILABILITY AND QSAR

EXCEPT FOR HAZARD IDENTIFICATION AND EXPOSURE TO SHORT-LIVED BENTHIC ORGANISMS, ALL THE REASONS REQUIRE A REASONABLE ESTIMATE OF STEADY-STATE TISSUE RESIDUES.

UNLESS THE RESULTS ARE SPECIFIC TO A SINGLE SPECIES, THE TEST SHOULD BE DESIGNED TO BE PROTECTIVE OF THE MAJORITY OF SPECIES.

EPA

GUIDANCE MANUAL: BEDDED SEDIMENT BIOACCUMULATION TESTS



U.S. Environmental Protection Agency
ERL-N
Pacific Ecosystems Branch
Bioaccumulation Team
Newport, OR

1989

Sediment Bioaccumulation Test
Key Procedures

---

1. 28-DAY EXPOSURE DURATION.

2. SEDIMENT-INGESTING ORGANISM REQUIRED.

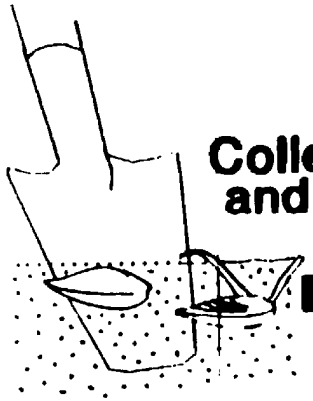
3. NO SUPPLEMENTAL FOOD USED.

4. SPECIES EXPOSED INDEPENDENTLY.

5. 80% OF STEADY-STATE TISSUE RESIDUES RECOMMENDED ACCURACY.

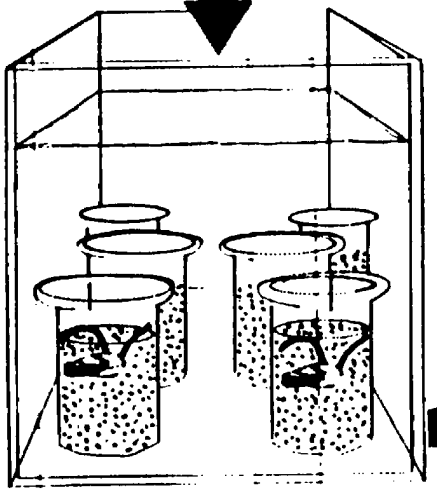
6. LONG-TERM TESTS OR TOXICOKINETIC APPROACHES USED FOR >80% ACCURACY OR SLOWLY ACCUMULATED COMPOUNDS.

Timeline for 28 Day Test



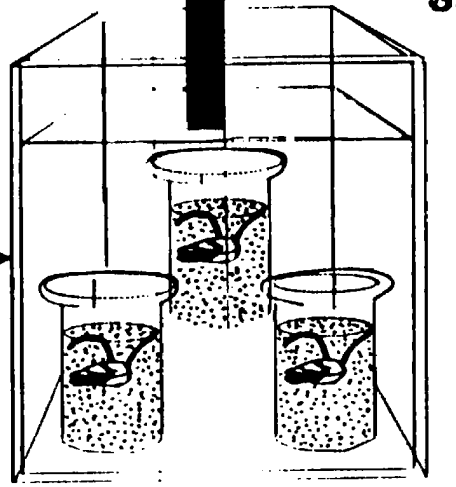
Collect Animals
and Sediment

Day 0
Sample -
Tissue
Sediment



Setup Experiment
(N=8)

Day 28
Sample -
Tissues
Lipids
Sediments



Tear Down

Criteria for Organisms Selection

1. Sediment Ingester*
2. Infaunal (preferably non-tubicolous)
3. Hardy
4. Easily Collected or Cultured
5. Sufficient Biomass for Chemical Analysis
6. High bioaccumulation potential
7. Feeding behavior understood
8. Suitable for mechanistic/kinetic studies

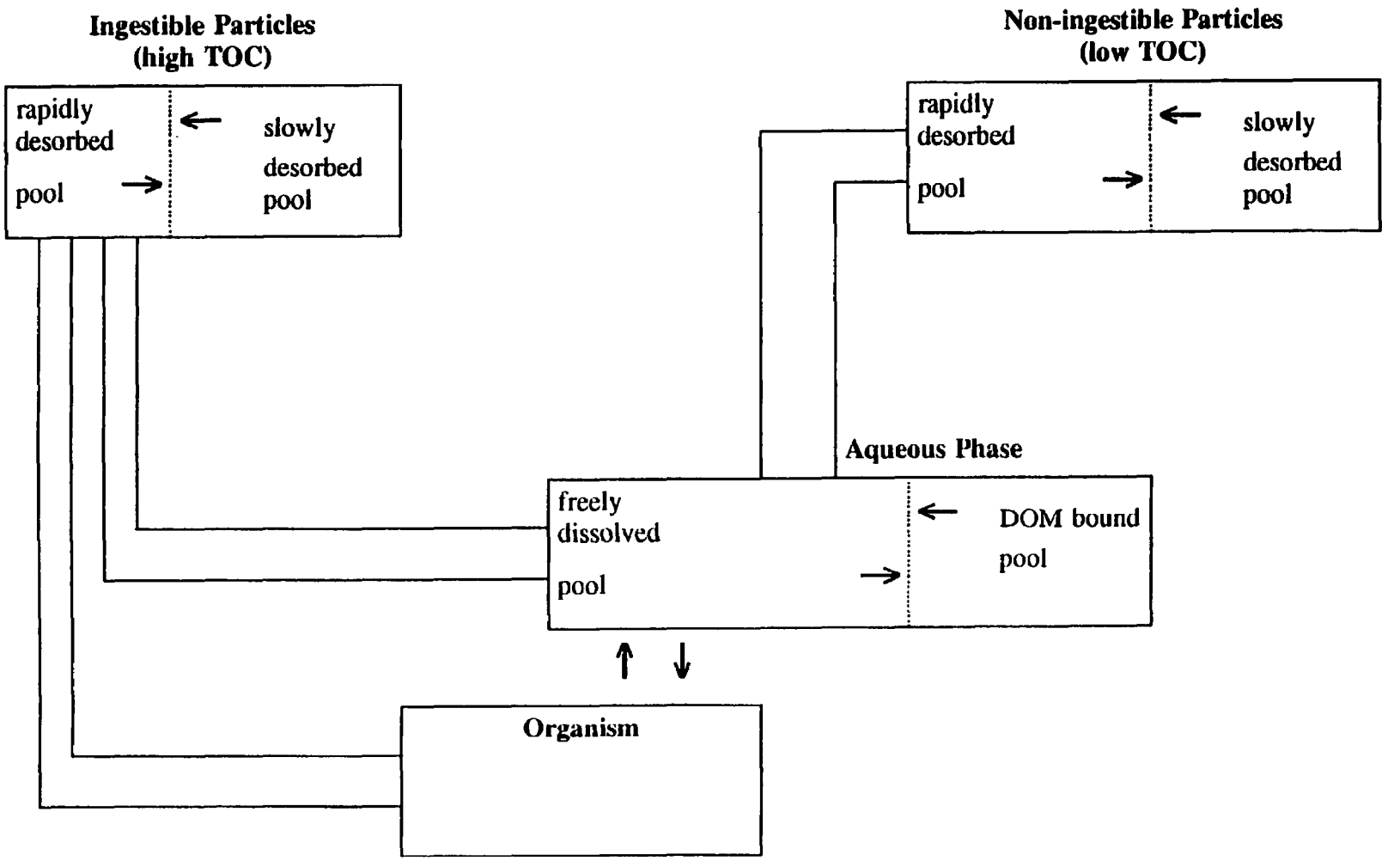
Table VII-1
PERTINENT CHARACTERISTICS OF TEST SPECIES

TAXA	Feeding Type	Biomass	$S^0/_{00}$	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	+?	>25	+	++	+	++
Nereis virens*	O/SDF	++	>10	++	-	+	++
Nereis diversicolor*	O/SDF	++	>10	++	-	+	++
Nucula spp.	SSDF	+	?	+	-	-	+
Palaemonetes pugio	SDF	+?	>10	-?	+	+	++
Yoldia limatula*	SSDF	+	>25	+	-	-	+

SDF = Surface Deposit Feeder
SSDF = Subsurface Deposit Feeder
IFF = Infaunal Filter Feeder
Fun = Funnel feeder
O = Omnivore
Pred = Predator
+ = good, sufficient
++ = very good
- = poor, insufficient

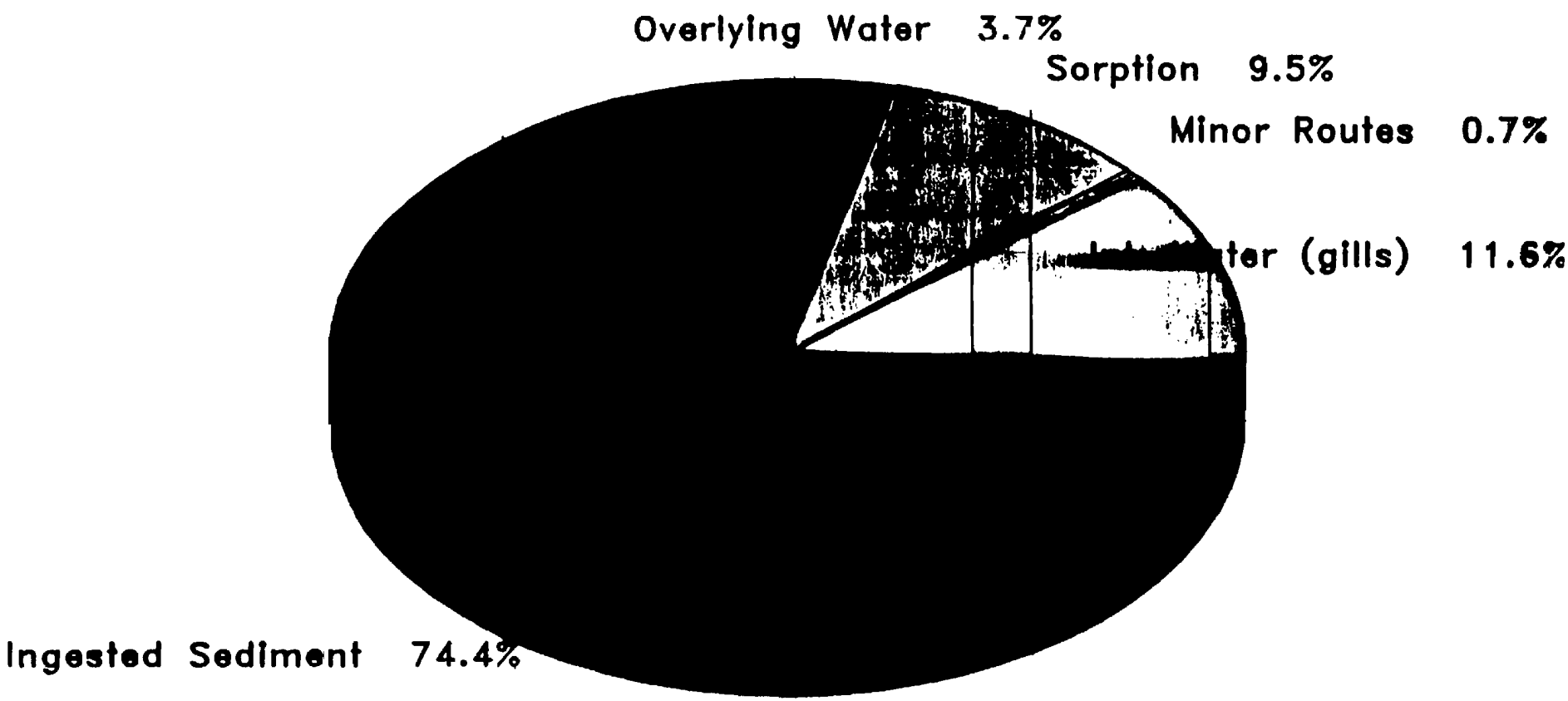
Bio Info. = Information on bioaccumulated toxicity

* Recommended test species
? = Tentative


Ingestible Particles
(high TOC)
rapidly
desorbed
pool
slowly
desorbed
pool
Non-ingestible Particles
(low TOC)
rapidly
desorbed
pool
slowly
desorbed
pool
Aqueous Phase
freely
dissolved
pool
DOM bound
pool
Organism

Uptake Routes of Hexachlorobenzene from Sediment

Macoma nasuta in bulk sediment



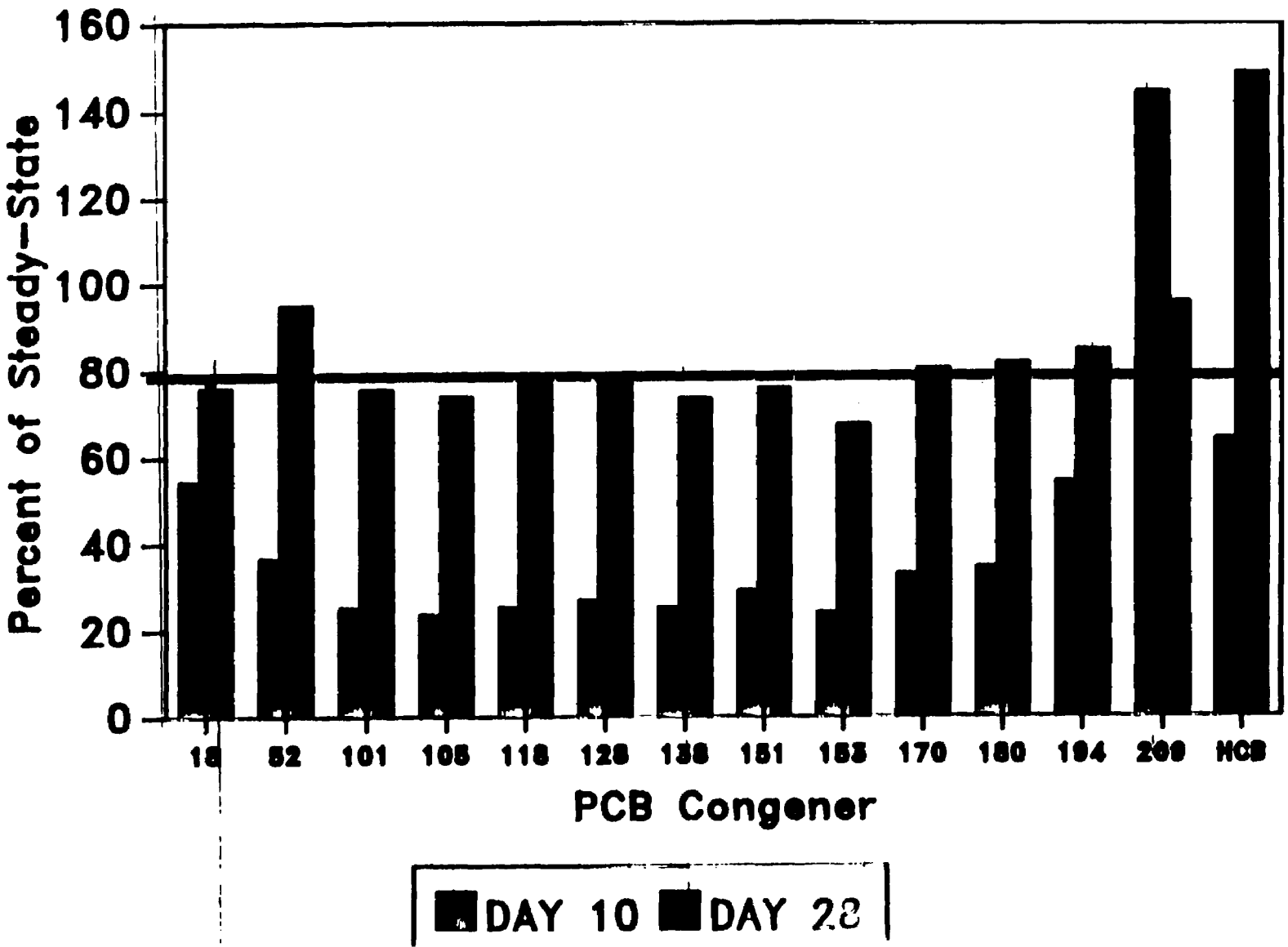
Increase in No. of Significant Differences in Tissue Residues With 10 and 28 Day Tests

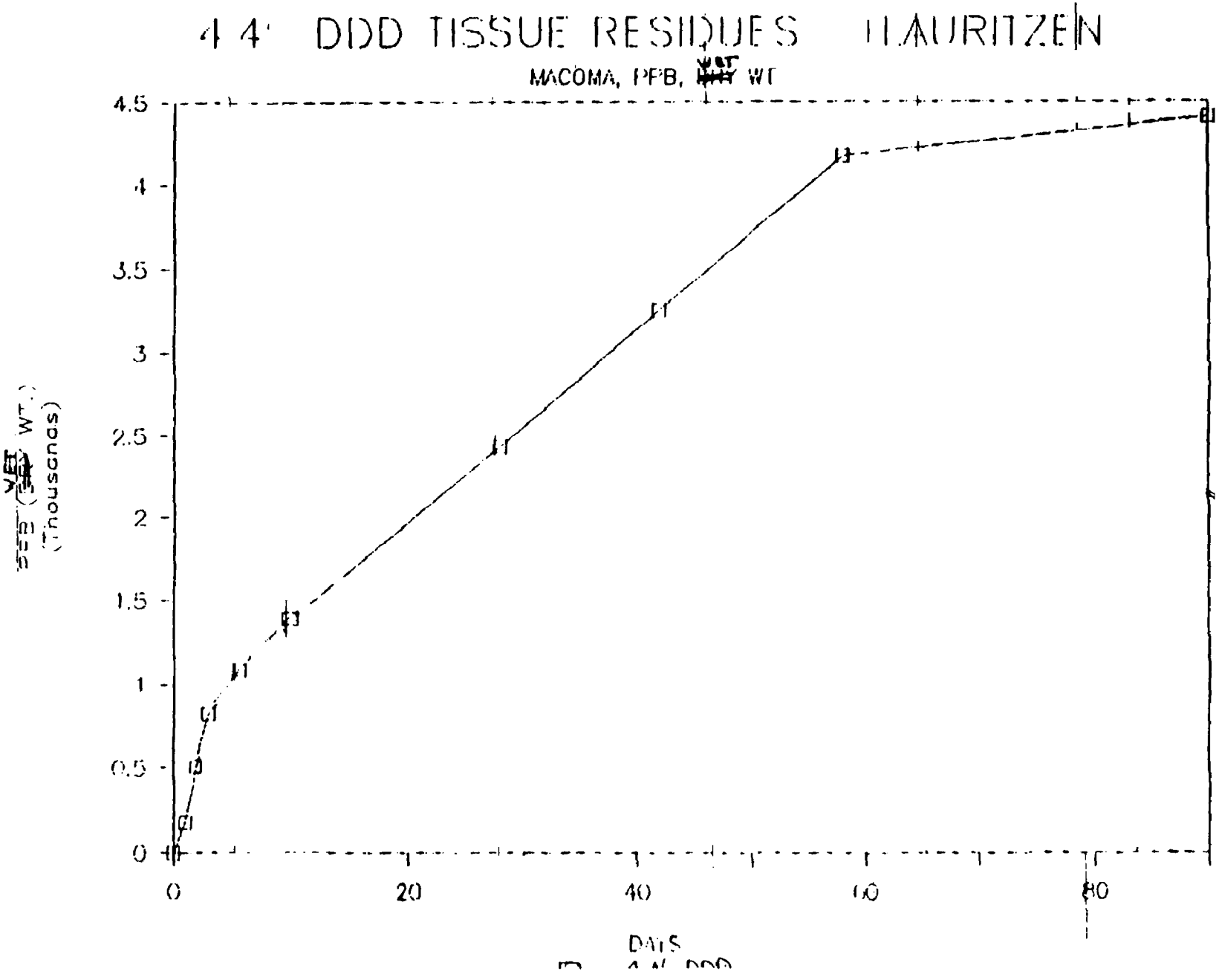
	Mercenaria Filter Feeder 10 Days	Nereis Deposit Feeder 10 Days	Nereis Deposit Feeder 28 Days	Macoma Deposit Feeder 28 Days	Add. Diff Detected to Macoma
PCBs	0/0	7/26	23/29	7/18	1/1
Pesticides	3/5	14/18	14/18	11/15	2/4
Metals	0/1	0/2	2/5	1/12	1/8

#/# = No. Sig. Diff. with 2-fold Diff. / No. Sig. Diff.

Data from Tracey et al., 1991

% STEADY-STATE IN 10 AND 28 DAY TESTS




4,4' DDD TISSUE RESIDUES IN AURITZEN
MACOMA, PPB, WET WT
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
DAYS

Statistical Recommendations for Conducting Bedded Sediment Bioaccumulation Tests

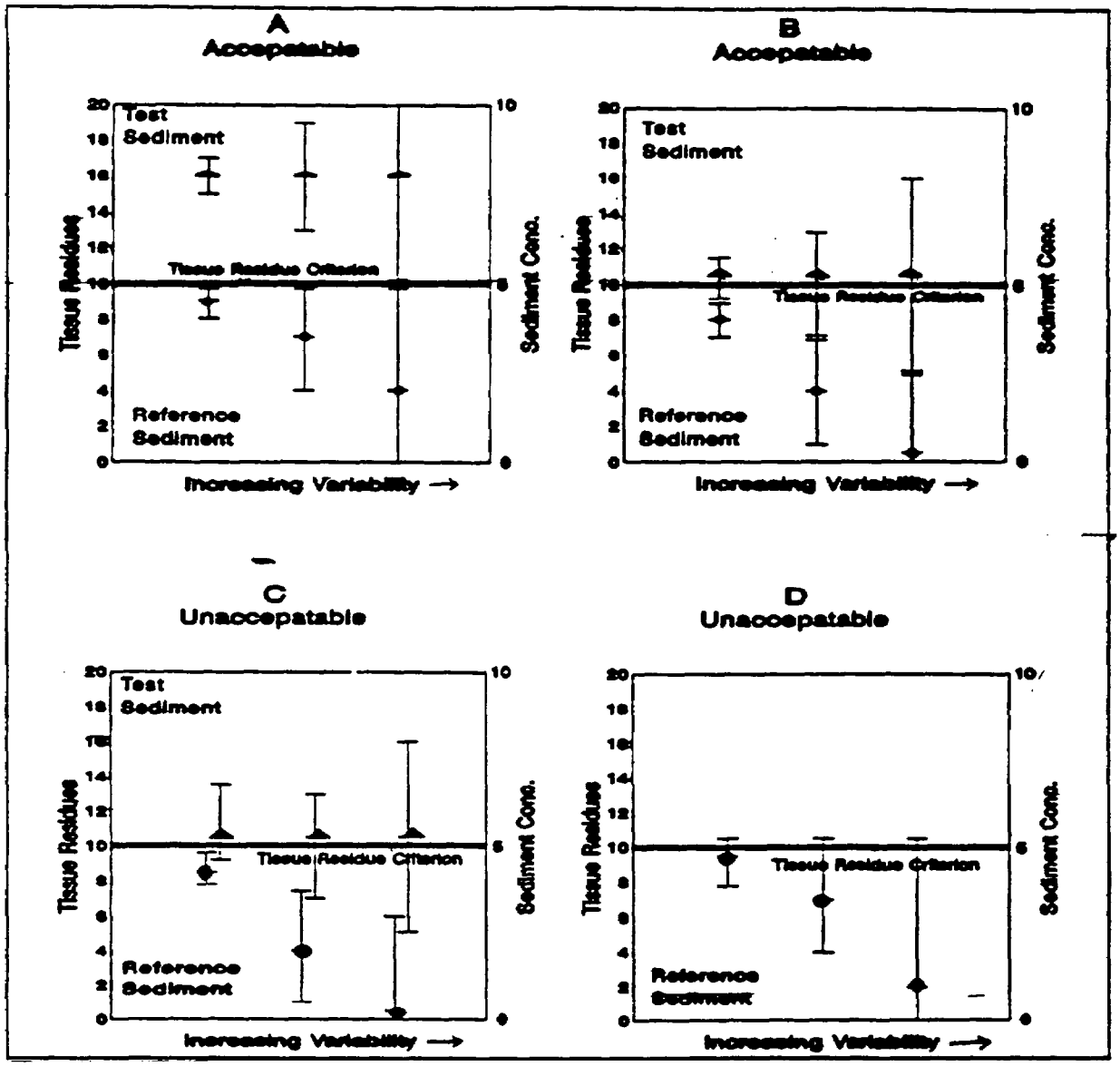
Alpha (type I error) = 0.05

Beta (type II error) = 0.20 or 0.05
or
Power = 0.80 or 0.95

Minimum detectable difference = 2-fold

To compare Control vs. Reference sediments and Reference vs. Test sediments, conduct one-tailed test.

For multiple Test sediments, the Type I error will be either comparison-wise or experiment-wise depending on site conditions.

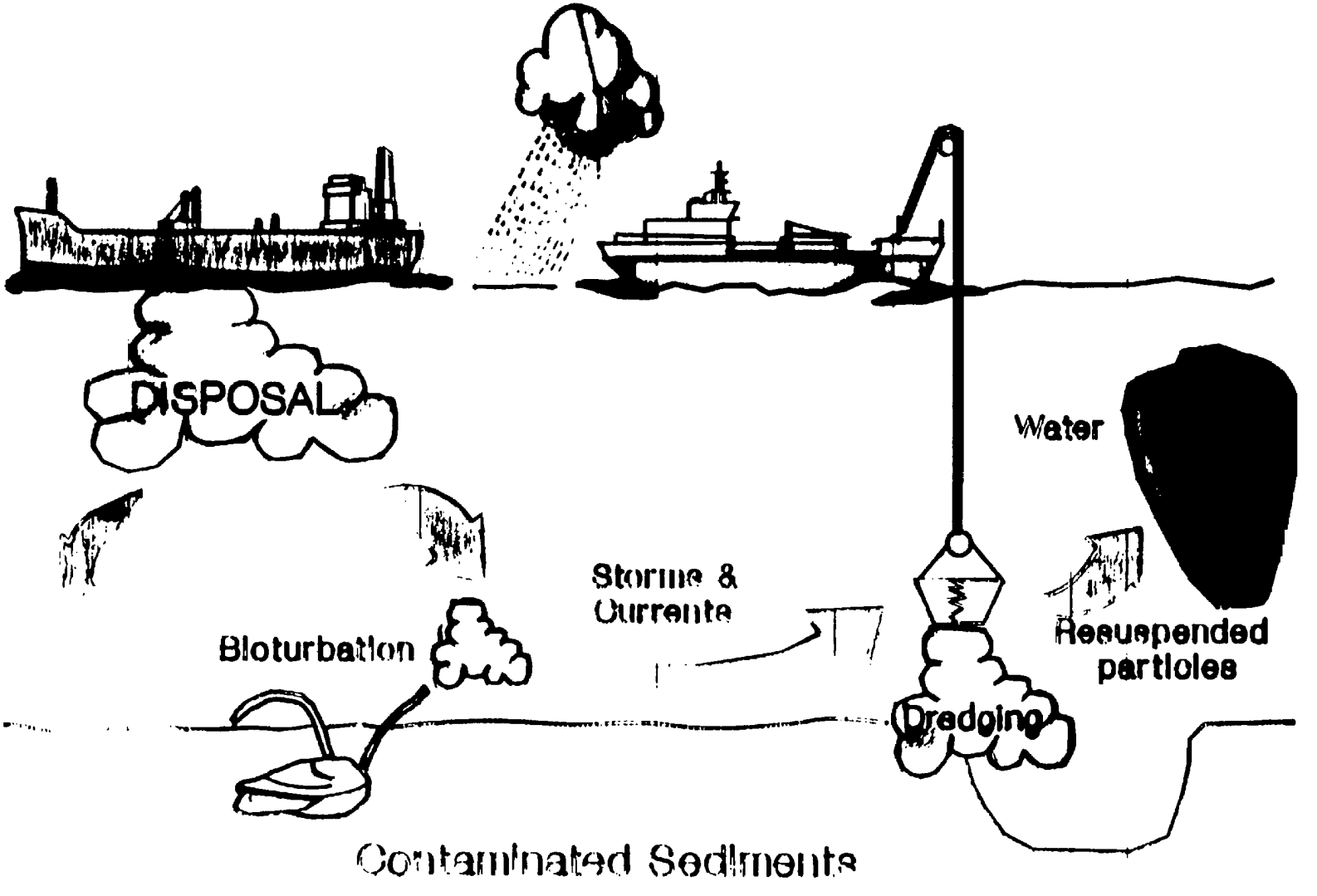


Appendix Figure 1: Acceptable and Unacceptable Reference Sediments. (A) Acceptable reference sediment with test confidence intervals not bracketing the tissue criterion. (B) Acceptable with test confidence intervals bracketing the tissue criterion. (C) Unacceptable because reference confidence intervals overlap the confidence intervals of a mean test residue exceeding the criterion. (D) Unacceptable because reference confidence intervals exceed the tissue criterion (test confidence intervals immaterial). The points represent the tissue residues that would result in organisms exposed to a particular sediment concentration. A BAF of 2 was used for illustrative purposes only.

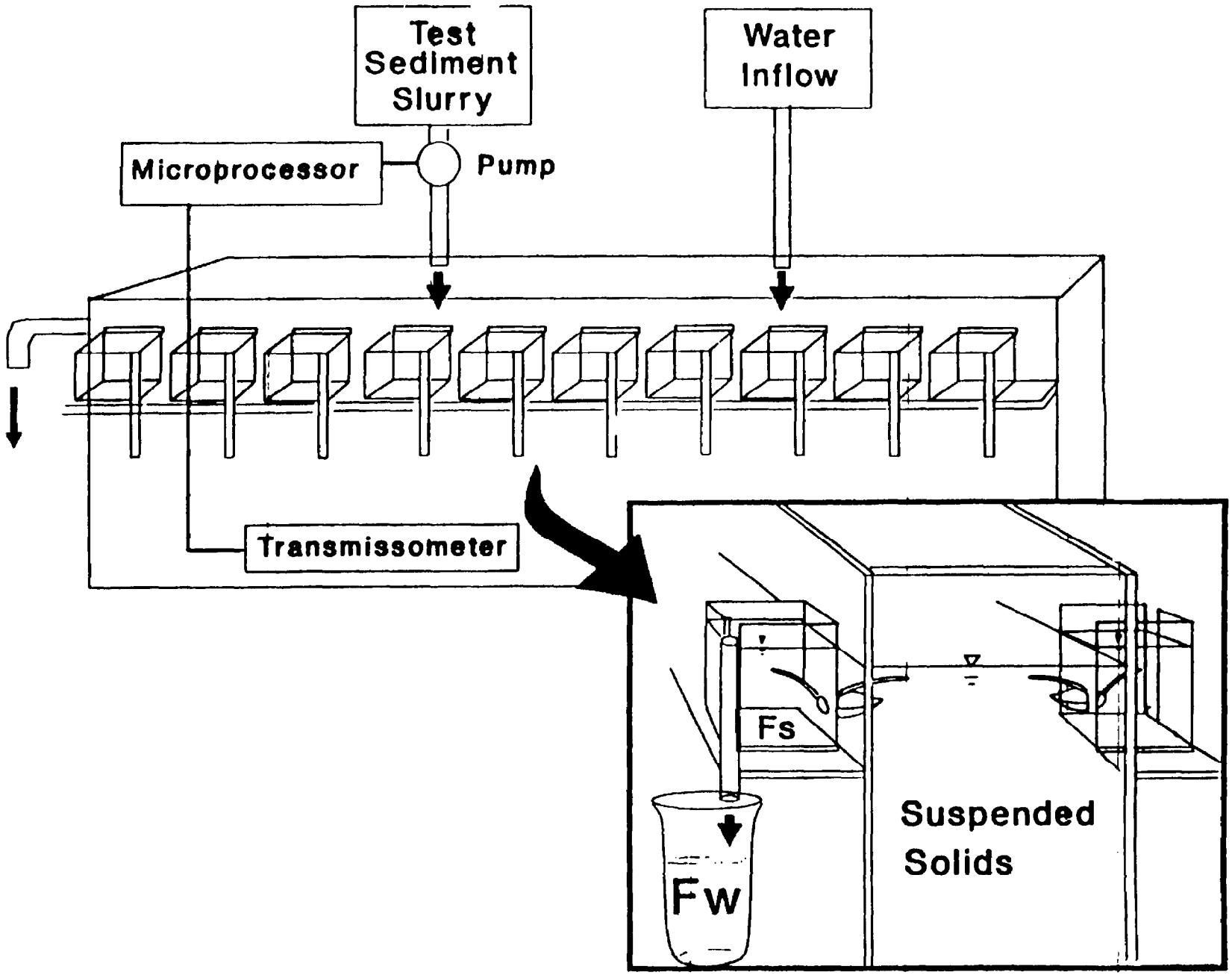
Green Book vs.
"Guidance Manual" and "Synthesis of Methods"

DREDGE MANUAL	GUIDANCE/SYNTHESIS DOCUMENTS
EqP BIOACCUMULATION MODEL SCREEN FOR NEUTRAL ORGANICS	EqP SCREEN FOR NEUTRAL ORGANICS BAF'S SCREEN FOR METALS/POLAR
"THEORETICAL BIOACCUMULATION POTENTIAL"	EQUIVALENT TO USING EqP BIOACCUMULATION MODEL WITH AF = 4
GOAL OF TEST = BIOACCUMULATION POTENTIAL"	GOAL OF TEST = TISSUE RESIDUES >= 80% OF STEADY STATE RESIDUES
10 DAYS FOR METALS 28 DAYS FOR ORGANICS	28 DAYS FOR ALL COMPOUNDS AS "STANDARD" SINGLE POINT ESTIMATE
N = 5	REPLICATION (n~ =8) BASED ON: TYPE I ERROR = TYPE II ERROR DETECT 2-FOLD DIFFERENCE
INCLUDES FILTER-FEEDING ORGANISMS	ONLY SEDIMENT-INGESTING ORGANISMS
T_0 CONTROL SAMPLES ANALYZED IF "DISCREPANCIES"	T_0 CONTROL SAMPLES ALWAYS ANALYZED
ALWAYS PURGE FOR 24 HOURS	PURGE FOR 24 HOURS EXCEPT FOR TROPHIC TRANSPORT STUDIES AND RAPIDLY METABOLIZED COMPOUNDS
CONDUCT TIER IV KINETIC OR FIELD EVALUATION ONLY IF FAIL TIER III	KINETIC, LONG-TERM EXPOSURES, OR FIELD STUDY DEPEND ON GOALS, COMPOUNDS, RESOURCES, AND ACCURACY
KINETIC TEST OR FIELD EVALUATION IF FAIL TIER III	(FOCUS ON EFFECTS OF TISSUE RESIDUES RATHER MORE ACCURATELY ESTIMATING TISSUE RESIDUES)
KINETIC APPROACH WITH k1 AND k2 ESTIMATED FROM UPTAKE CURVE ONLY	KINETIC APPROACH WITH kS (=k1) AND k2 ESTIMATED INDEPENDENTLY
NO QUANTITATIVE CRITERIA FOR CONTROL SEDIMENTS	GUIDELINES FOR ACCEPTABLE SEDIMENT POLLUTANT CONC. IN CONTROLS
NO QUANTITATIVE CRITERIA FOR REFERENCE SITES	STATISTICAL CRITERIA BASED ON OVERLAP WITH TISSUE RESIDUE "CRITERIA"

RESUSPENSION EVENTS



Resuspension Exposure Apparatus



BEDDED SEDIMENT BIOACCUMULATION TEST RESEARCH NEEDS

I. INTERLABORATORY ROUND ROBIN

II. FIELD VALIDATION

III. ADEQUACY OF 28 DAY TESTS

IV. REFINEMENT OF CULTURING AND EXPOSURE METHODS FOR STANDARDIZED SPECIES
    1. BIOLOGICAL: TEMPERATURE, SALINITY, GRAIN SIZE, TOC
    2. EXPOSURE: SEDIMENT/ORGANISM MASS, GUT PURGING

V. EFFECTS OF SEDIMENT COLLECTION, STORAGE, AND SPIKING ON BIOAVAILABILITY

VI. "STANDARDIZATION" OF LIPID METHOD

VII. TESTING/GUIDANCE FOR NEED FOR 2 BIOACCUMULATION TEST SPECIES

VIII. DEVELOPMENT OF REFERENCE BIOACCUMULATION SEDIMENT

IX. REFINEMENT OF STATISTICAL DESIGNS

X. DEVELOPMENT/VALIDATION OF KINETIC ALTERNATIVES TO 28-DAY TEST

XI. TEST SPECIES FOR SUBTROPICAL SUBARCTIC, AND OLIGOHALINE HABITATS

BIOAVAILABILITY OF SEDIMENT CONTAMINANTS RESEARCH NEEDS

I. QUANTIFY UNCERTAINTY/ASSUMPTIONS OF EQUILIBRIUM PARTITIONING BIOACCUMULATION MODEL

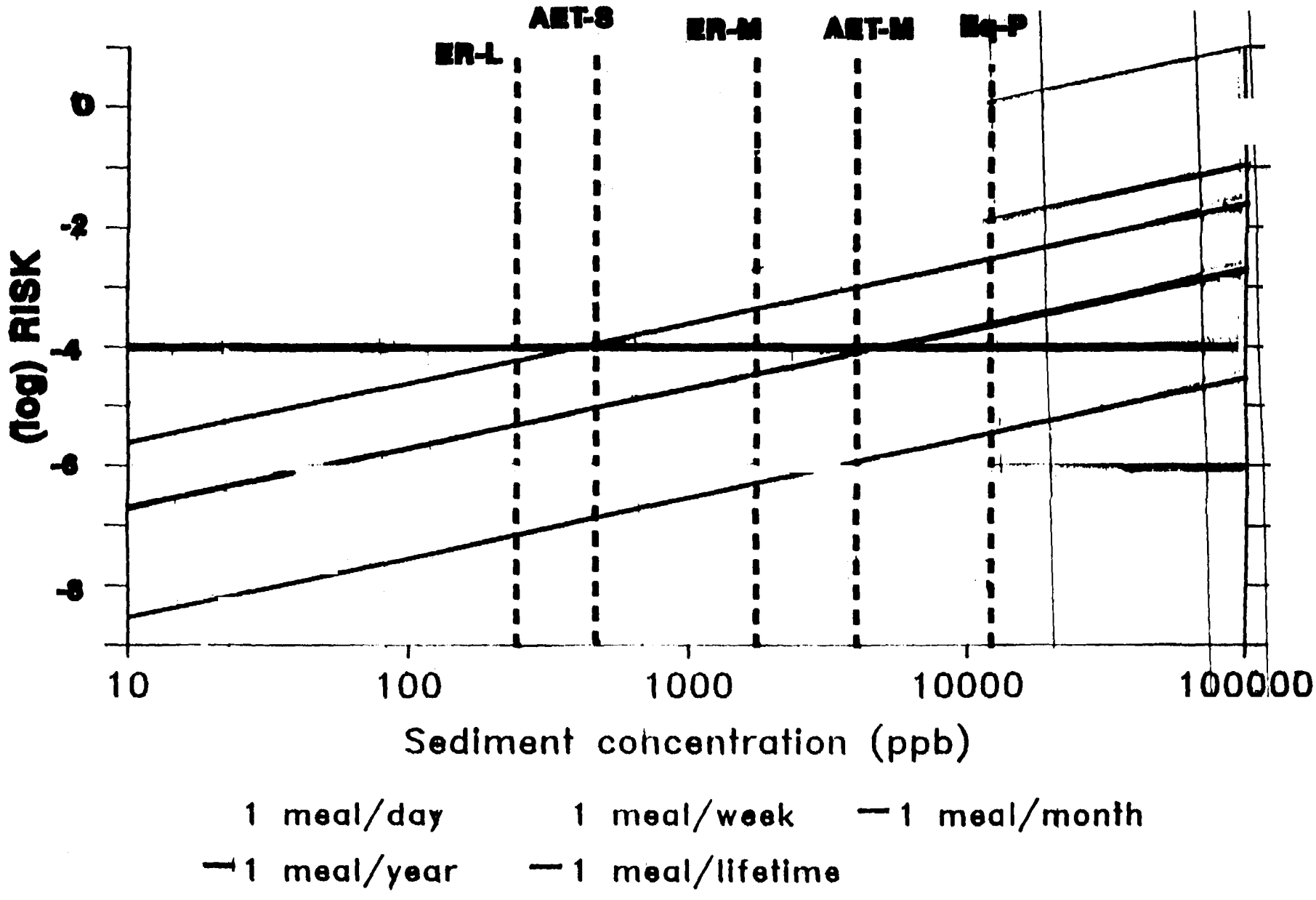
II. DEVELOP SCREENING MODEL/APPROACH FOR METALS

III. DEVELOP METHODS TO QUANTIFY EXPOSURE, INCLUDING INGESTED DOSE, OF BENTHIC SPECIES

IV. DEVELOP/VALIDATE TOXICOKINETIC APPROACHES FOR BENTHIC SPECIES

V. COUPLE TOXICOKINETIC AND TOXICODYNAMIC APPROACHES TO PREDICT TISSUE RESIDUE EFFECTS ON BENTHOS

VI. PREDICT TISSUE RESIDUE EFFECTS FROM "WEIGHT OF EVIDENCE" OR USE AS INDEPENDENT VARIABLE IN AET'S


Cancer Risk
B(a)P
ER-L
AET-S
ER-M
AET-M
Eq-P
(log) RISK
0
-2
-4
-6
-8
10
100
1000
10000
100000
Sediment concentration (ppb)
1 meal/day
1 meal/week
1 meal/month
1 meal/year
1 meal/lifetime

Discussion of the Use of Lumbriculus variegatus in Freshwater Sediments

Gary Ankley, U.S. EPA Environmental Research Laboratory - Duluth, MN

I. Desirable Attributes in Selection of Species for Sediment Bioaccumulation Testing

A. Readily available
B. Known exposure history
C. Adequate tissue mass for trace analyses
D. Easily handled
E. Amenable to long-term exposures
F. Reflect concentrations of contaminants in field organisms (i.e., exposure is realistic)
G. Tolerant of a wide range of physico-chemical conditions in sediments (e.g., particle size)

II. Freshwater Species Uses for Sediment Bioaccumulation Testing

A. Chironomids
B. Amphipods
C. Mayflies
D. Clams
E. Fishes
F. Oligochaetes

III. Oligochaetes as Bioaccumulation Test Species

A. Certain species easily cultured and, therefore, readily available with known exposure history
B. Provide adequate tissue mass for trace residue analysis
C. Can be used in long-term exposures
D. Easily handled and tolerant of a wide range of physico-chemical conditions
E. Realistic exposure to sediment-associated contaminants

IV. Attributes of Lumbriculus variegatus

A. Relatively large (~5-10 mg/organism)
B. Easily handled
C. Tolerant of wide range of physico-chemical conditions (e.g., DO, particle size)
D. Tolerant of many contaminants
E. Can be used in long-term tests
F. Standard culture and test methods have been developed
G. Some field validation

V. Lumbriculus variegatus in Aquatic Toxicological Studies

A. Single chemical toxicity testing (Bailey and Liu, 1980; Ewell et al., 1986; Nebeker et al., 1989)
B. Sediment toxicity testing (Ankley et al., 1991a; 1991b; Call et al., 1991; Carlson et al., 1991; Phipps et al., 1992; West et al., 1993; Dermott and Munawar, 1993)
C. Metal bioaccumulation studies (Ankley et al., 1991b; 1993; Carlson et al., 1991)
D. Nonionic organic bioaccumulation studies (Ankley et al., 1992; Call et al., 1991; Nebeker et al., 1989; Schuytema et al., 1990)

Identification of Long Term Needs for Assessing Sediments

Norm Rubinstein, U.S. EPA Environmental Research Laboratory - Narragansett, RI

I. Major Goals

A. Identify "problem" sediments

B. Assess potential impact of contaminated sediments on aquatic habitats, wildlife, and human health

C. Remediate contaminated sediment sites in a cost-effective and environmentally consistent manner

II. Scientific Questions and Research Needs

A. What are the most technically valid and cost-effective approaches for deriving sediment quality criteria?

B. How can we best identify and quantify contaminated sediment exposure regimes?

C. What are the key physico-chemical factors controlling the biological availability of sediment-associated contaminants?

D. What is the ecological and human health significance of sediment mediated tissue residues in aquatic food chains?

E. What kinds of assessment methods are needed to best identify ecologically relevant endpoints and how can these techniques fit within a tiered testing strategy for eco-risk assessment?

F. Which specific fractions or individual constituents of sediment-associated pollutants are of significant toxicological concern?

G. What are the best approaches for identifying and cleaning up contaminated sediment sites?

H. To what extent is natural recovery sufficient to remediate contaminated sites?

III. Research Areas

A. Sediment quality criteria

B. Contaminated sediment assessment methods

C. Remediation technology

D. Monitoring

IV. Sediment Quality Criteria (SQC)

A. Field validation of EqP approach

B. SQC development for ionizable organic and metallics (e.g., AVS approach)

C. Development of SQC tissue residue approach to address wildlife and human health concerns

V. Exposure Assessment

A. Exposure assessment modeling for aquatic disposal of dredged materials

B. Wasteload allocation models to evaluate contaminated sediments and source control options

C. Chemical analytical methods development

VI. Effects Assessment

A. Development and validation of acute and chronic testing protocols for contaminated sediments in freshwater and marine systems

B. Development and validation of contaminated sediment bioaccumulation test methods

C. Development of tissue residue thresholds

D. Development of trophic transfer models for sediment mediated tissue residues

VII. Remediation Methods

A. Development and validate methods for in situ containment and treatment of contaminated sediments

B. Develop methods to examine rates of natural recovery for benthic communities

VIII. Ecological Risk Assessment

A. Develop methods to integrate stress response relationships for ecologically relevant endpoints

Contaminated Sediment Research Issue

- Major Goals:
  - Identify "problem" sediments
  - Assess potential impact of contaminated sediments on aquatic habitats, wildlife, and human health
  - Remediate contaminated sediment sites in a cost-effective and environmentally consistent manner

ORD Issue Based Planning System

- Major Components
  - ORD Strategic Plan
  - Research Issue Strategies
  - Issue Research Plan
- Participants
  - Issue Planner
  - Planning Groups
  - Research Strategy Council
  - Research Committees

ORD Issue Based Planning System

- Objectives
  - focus on high risk environmental problems
  - multi-media approach
  - top-down direction by senior EPA management
  - integration with the Agency planning process
  - promote greater interaction with the science community
  - simplify the research planning process

Break-Out Workgroup for Freshwater Sediment Issues: Overview of the Day

Gary Ankley, U.S. EPA Environmental Research Laboratory - Duluth, MN

I. General Discussion Format

A. Brief presentation of survey results on culturing and testing
   1. Hyalella azteca
   2. Chironomus tentans
   3. Lumbriculus variegatus

B. Discussion of major culturing and testing issues
   1. List of proposed issues (by organism)
   2. Resolution (if possible) of issues

II. Selection of Test Species

A. Current and historical technical acceptance of test organisms
B. Logistical concerns (e.g., organism availability)
C. Availability of some test methods

III. Survey Techniques

A. Questionnaires on culturing/testing distributed to workshop participants and others testing target species
B. Focused on H. azteca, C. tentans/riparius, L. variegatus, but other species identified as well
C. Results summarized by issue

IV. General Culturing Issues

A. Substrate
B. Genetic drift/stream differences
C. Density
D. Known age systems
E. Water
F. Nuisance organisms
G. Flow-through vs. static
H. Light/photoperiod
I. Feeds/feeding
J. Temperature
K. QA/QC (e.g., reproduction, reference toxicants)

V. General Testing Issues

A. Test lengths/endpoints
B. Organism age
C. Water renewal (volumes, frequency, method)
D. Physical test system (sediment volume, etc.)
E. Test condition and design (chambers, lighting, etc.)
F. Interpretation of sediment variables (e.g., organic carbon particle size) on test results
G. Feeds/feeding regimes
H. QA/QC (for acceptable test)

NOTE: For discussion purposes, the proposed issues initially were ranked based upon:

- Immediacy of issue
- Generality across tests

SURVEY RESPONDENTS

Institution	*H. azteca*	*C. tentans/riparius*	*L. variegatus*
Columbia FWS	X	X	X
Athens FWS	X	X	
Duluth EPA	X	X	X
University of WI-Superior	X	X	X
NOAA (Ann Arbor)			X
Wright State	X	X	
ABC Laboratories	X	X	X
Environment Canada	X	X	
EVS Consultants	X	X	
Region 8 EPA	X		
Old Dominion	X		
Cincinnati EPA	X	X	
Region 1 EPA	X	X	
University of Mississippi	X	X	
Michigan State University	X	X	
Maryland DE	X		
Canada Oceans & Fishes	X		
Miami University	X		
Washington State DE	X		

Development of a Standard Protocol for Testing Hyalella azteca

Teresa Norberg-King, U.S. EPA Environmental Research Laboratory - Duluth, MN

I. **Summary of Culture Methods Used as Reported in Questionnaires**

II. **Summary of Test Methods Used as Reported in Questionnaires**

III. **Proposed Key Issues for Discussion**

RANKED ISSUES FOR CULTURING *H. AZTECA*

- **Known Age Systems**
- **Feeds/Feeding Regimes**
- **Water** (Reconstituted vs. Surface Waters)
- **Flow-through vs. Static**
- **QA/QC** (e.g., Reproduction, Reference Toxicants)
- **Genetic Drift/Strain Differences**
- Substrate
- Density
- Nuisance Organisms
- Light/Photoperiod
- Temperature
- Other _____

RANKED ISSUES FOR TESTING *H. AZTECA*

- **Test Lengths/Endpoints**
- **Organism Age**
- **Water Renewal**
  (Volumes, Frequency, Method)
- **Interpreting Effects of Sediment Variables on Test Results**
  (e.g., organic carbon, particle size)
- **Feeding in Tests**
- **QA/QC (Criteria for Acceptable Test)**
- Test System
  (e.g., Chamber Size, Construction, Replicates, Temperature, Light/Photoperiod, et cetera)
- Water and Sediment Quality Monitoring
- Sediment Volumes (Physical test system)
- Other _____

Frequency of Culture Re-starts

1 month	1
2 month	6
3 month	1
4 month	1
continuous	1
quarterly	2
2×/year	1
variable	1

Variety of Foods Tried (listed singly)

yeast
Cerophyll®
wheat grass
Chlorella
diatom (*Spirulina*)
alfalfa grass
Tetramin®
Nutrafin®
YCT
paper towels
S. capricornutum
Ankistrodesmus
maple leaves
paper towels innoculated with Tetramin®
rabbit food
brine shrimp
astroturf
aquatic plants
sediment

Feeding Frequency in Cultures

Flow-through cultures

3×/wk	1
1×/d	1
1×/wk	1

Static cultures

1×/month	1
3×/wk	3
2×/wk	8
1×/wk	2
1×/day	1
2×/day	1
as nec	1

Waters Used for Culturing

tap	7
well	4
surface	3
reconstituted	3
mix of sources	1

Foods fed Cultures of:

Laboratory	Food(s) Using	How Long
ABC Lab.	Yeast, Cerophyll®, *Chlorella,* wheat grass, diatom, alfalfa	<1 y
Dept Fish/Oceans	Tetramin® flakes	5-6 y
Environ. Canada	Nutrafin® flakes	~1 y
EPA-Duluth	YCT & diatoms	~1 y
EPA Region 1	rabbit pellets	~1 y
EPA Region 8	paper towels & flake fish food	~1 y
EPA Newtown	Cerophyll® & *S. capricornutum*	3 mo
EVS Consultants	YCT and *S. capricornutum*	2 y
MD Dept. Env.	Tetramin® & leaves	~3 y
Miami Univ.	digested paper towels inoculated with Tetramin®	2 y
Mich. State	Tetramin®	1.5 y
NFCRC-Athens	leaves	5 y
NFCRC-Columbia	leaves & Tetramin®	5 y
Old Dominion	leaves & rabbit chow	2 y
State of Wash.	rabbit food & leaves	5 y
Univ. of Miss.	leaves & rabbit chow	3 y
Univ. of WI-Sup.	YCT & *Ankistrodesmus*	6 mo
Wright State	rabbit pellets, Tetramin®	4 mo

Reconstituted Water

- 7 labs have used it
- 4 with good success

Characteristics of Culture Water (n = 18)

very soft	1
soft	5
moderately hard	7
hard	4
very hard	1

Types of Water Renewal in Cultures

flow-through	3
static, static renewal	14

Culture Records Desirable/Maintained

Parental survival	56%
Age brood animals started	50%
Routine chemistries	94%
Quality of food	69%
Freq. of culture initiations	100%

Reference Toxicants Used (n = 14)

cadmium	6
copper	3
KCl	5
NaCl	1
zinc	1
chromium	1

Types of Substrates Currently in Use

plastic mesh	2
gauze	4
nitex	1
sediment/towels	1
towels	2
sand/towels/nitex	1
plastic/leaves	1
leaves	4
mesh/towel	1
none	1

Substrates Used

Laboratory	Choice	Others Tried
ABC Lab.	nylon mesh	maple leaves
Dept Fish/Oceans	sterile gauze	aquatic plants, none, nitex, leaves, astroturf
Environ. Canada	gauze	sediment
EPA-Duluth	gauze	leaves, sediment
EPA Region 1	plastic mesh pad, leaves	leaves only
EPA Region 8	sediment	--
EPA Newtown	kraft paper towel	leaves
EVS Consult.	silica sand, leaves, nitex cones	gauze
MD Dept. Env.	leaves	--
Miami Univ.	paper towels (unbleached)	--
Mich. State	gauze, unsterilized towel strips	--
NFCRC-Athens	leaves	--
NFCRC-Columb	leaves & 3M plastic web	--
Old Dominion	leaves	--
State of Wash.	leaves	--
Univ. of Miss.	leaves	--
Univ. of WI-Sup.	gauze	--
Wright State	leaves, paper towels polyethylene mesh	--

Culture Temperature (°C)

15	1
20	3
23	8
25	4
19-23	1

Culture Chambers Used

Aquaria Sizes	**1 L - 39 L**
Water Volume	**0.75 L - 38 L**

Test Lengths

96 h	3
7 d	4
10 d	8
10-14 d	1
14 d	4
20 d	1
28 d	4

Test Age

known age	7
sieve for size/age	8
mixed age	2
unknown	1

Water Renewal and Frequency

Static		
	no water replacement	10
	top off	2
Renewal		
	4-6 h	1
	24 h	2
	72 h	1
	1.5 addn/d	1
	4 addn/d	2
	2×/wk	1
	not specified	1

FEEDING IN SEDIMENT TESTS

7×/wk	5
3×/wk	5
2×/wk	2
1×/wk	1
every 48 h	1
initiation only	1
none	1

Test Acceptability Criteria

Survival

60%	1
70%	2
80%	13
90%	1

What is Reasonable to Evaluate
Test Acceptability

Survival

yes	18
no	0

Minimum growth

yes	3
no	8

Reference Toxicity Test

yes	7
no	7
maybe	1

Test Temperatures (°C) (n = 17)

20	6
25	5
23	4
20	1
20-25	1

VARIOUS SOURCES OF *H. azteca*

St. Louis River Strain

EPA Duluth (89)
Univ. of WI-Superior (91)

Burlington Strain

Dept Fish & Ocean (85)
Environment Canada (91)

Michigan State Pond Strain

Michigan State (90)

Nebeker Strain

NFCRC-Columbia (87)

ABC Laboratories (88)
Maryland Dept Environ. (90)
NFCRC-Athens (87)
Old Dominion (90)
Univ. of Mississippi (89)

State of Washington (87)

EVS Consultants (90)

EPA Newtown (90)

EPA Region 1 (91)
EPA Region 8 (--)
Miami University (90)
Wright State (91)

Development of a Standard Protocol for Chironomus tentans

Robert Hoke, ASCI - Duluth, MN

I. Summary of Culture Methods Used as Reported in Questionnaires

II. Summary of Test Methods Used as Reported in Questionnaires

III. Proposed Key Issues for Discussion

SURVEY RESULTS

Chironomus tentans (riparius) Culture Conditions

No. of Responses - 8 (2)

CULTURE TYPE	FT-2.SWR-8
TEMPERATURE	10-25° c
LIGHT QUAL./INT.	ambient lab fluor./50-120 ft-c
PHOTOPERIOD	16 L / 8 D
CULTURE CHAMBER SIZE	1 - 40 L
CHAMBER WATER VOLUME	1 - 30 L
CHAMBER WATER RENEWAL RATE	once/day - evaporative loss only
NO. OF CHAMBERS	4 - 40
NO. OF ORGANISMS/CHAMBER	50 - 800
CHAMBER RESTART INTERVAL	2X weekly - every 6 months
AGE OF RESTART ORGANISMS	egg cases - <24 h old larvae
ORGANISM REMOVAL	daily - as needed
FEEDING REGIME	bleached-1/unbleached-7/sand-2 (paper towels)
CHAMBER CLEANING	none - weekly
AERATION	yes - 10
CULTURE WATER	soft/moderately hard/very hard, lake-1/tap-3/well-5/recon-1

SURVEY RESULTS

Chironomus tentans (riparius) Test Conditions

No. of Responses - 8

TEST TYPE	S(6); R(3)-(4/d, 2X or 3x/wk)
TEST DURATION	10d(5),2-14d(1),4-1d(1),10-14d(1)
TEMPERATURE	20(2), 22(2), 23(2), 25(2), °C
LIGHT QUAL./INT.	ambient lab fluor./25-120 ft-c
PHOTOPERIOD	16 L / 8 D
TEST CHAMBER SIZE	50 ml(2), 250(2), 300, 1000(2), 2000
CHAMBER SEDIMENT VOLUME	10-200 ml
CHAMBER OVERLYING WATER VOLUME	40-1800 ml (1:4, S:W, 6 of 8)
NO. OF CHAMBERS (REPS/SAMPLE)	2-15; 3-4 (6), 15 (2)
NO. OF ORGANISMS/CHAMBER (REP)	15-80
AGE OF TEST ORGANISMS	0-16d; 10-14d (7)
SIZE OF TEST ORGANISMS	---
FEEDING REGIME	daily (5), 2X-3X weekly (3)
TEST CHAMBER CLEANING	none
AERATION	yes(6), no(2)--ERLD,UM
TEST WATER	soft/moderately hard/very hard, lake-1/tap-3/well-5/recon-1
TEST ACCEPTABILITY CRITERION	>40% saturation (2), temp. (1)
MINIMUM SURVIVAL - 7	70% (4), 75-80% (1), 80% (2)
MINIMUM LENGTH - 1/WEIGHT - 7	---

Chironomus tentans (riparius) CULTURE CONDITION ISSUES

- SUBSTRATE
- CULTURE WATER
- FOOD/FEEDING REGIME
- QUALITY ASSURANCE/QUALITY CONTROL

Chironomus tentans (riparius) TEST CONDITION ISSUES

- ORGANISM AGE
- TEST DESIGN (TYPE, RENEWAL, FEEDING)
- EFFECTS OF ABIOTIC FACTORS
- TEST LENGTH AND ENDPOINTS
- QUALITY ASSURANCE/QUALITY CONTROL

Issues for Lumbriculus variegatus

Peter Landrum, NOAA Great Lakes Environmental Research Lab - Ann Arbor, MI
Gary Ankley, U.S. EPA Environmental Research Laboratory - Duluth, MN

I. Culturing

- A. Substrate
- B. Density
- C. Water
- D. Feeding
- E. Temperature
- F. Light
- G. QA/QC

II. Testing

- A. Age
- B. Loading rates
- C. Test lengths
  - 1. Example
- D. Water renewal
- E. Sediment volume
- F. Interpreting effects of sediment variables

III. To Feed or Not To Feed?

IV. Sediment Avoidance - Effect on Exposure

V. Gut Purging for Bioaccumulation

CULTURING L. variegatus

Substrate - brown paper towels, soaked 48 h

Density - must be low enough to maintain water quality

Water - well or lake water

Flow-through or static - water quality is the main issue both have been used

Feeding - Augment paper towels with trout chow. The organisms can be over fed

CULTURING CONTINUED

Genetic drift/strain differences - ?

Known age systems - not practical

Light/photoperiod - no studies performed; ambient laboratory

Temperature - 22 - 24°C

QA/QC - monitor water quality; population doubling rate; reference toxicity

TESTING CONDITIONS

Organism Age - adults

Loading Rates - 10 - 100 gOC/g dry wt worm, Note: with very low organic carbon sediments additional feeding may be required to maintain health; for toxicity tests 20 mg trout starter every 5 d

Test Lengths - Bioaccumulation (steady-state for many compounds may be obtained in 7 to 10 days based on elimination kinetics for hexachlorobiphenyl); Organisms will avoid extremely contaminated sediment reducing their exposure.

Water Renewal - Static as needed to maintain water quality particularly dissolved oxygen; flow-through 4 changes per day

Sediment Volumes - Sufficient amount for burrowing and food supply

TESTING CONDITIONS CONTINUED

Interpreting Effects of Sediment variables on Test Results -

1. Organisms will not grow and reproduce well in very low organic carbon sediment no mater what the ratio of organisms to sediment

2. Organisms tend to not reproduce well when over fed

3. Organisms will reduce their exposure through avoidance when the sediments are highly contaminated - thus sediment concentration does not always reflect either effects or amount accumulated.

4. L. Variegatus is very sensitive to ammonia. Ammonia buildup must be avoided.

FEEDING

Expected impact of feeding:

1. Reduce exposure through preferential feeding on uncontaminated food and/or dilution of the organic carbon partitioned material

2. Enhanced elimination when fed uncontaminated food versus contaminated material

3. Feeding Selectivity can change the effective dose and relative accumulation

4. When feeding is a dominant route changes in organism health due to toxicant effects can reduce ingestion rate and therefore, exposure.

GUT PURGING L. variegatus

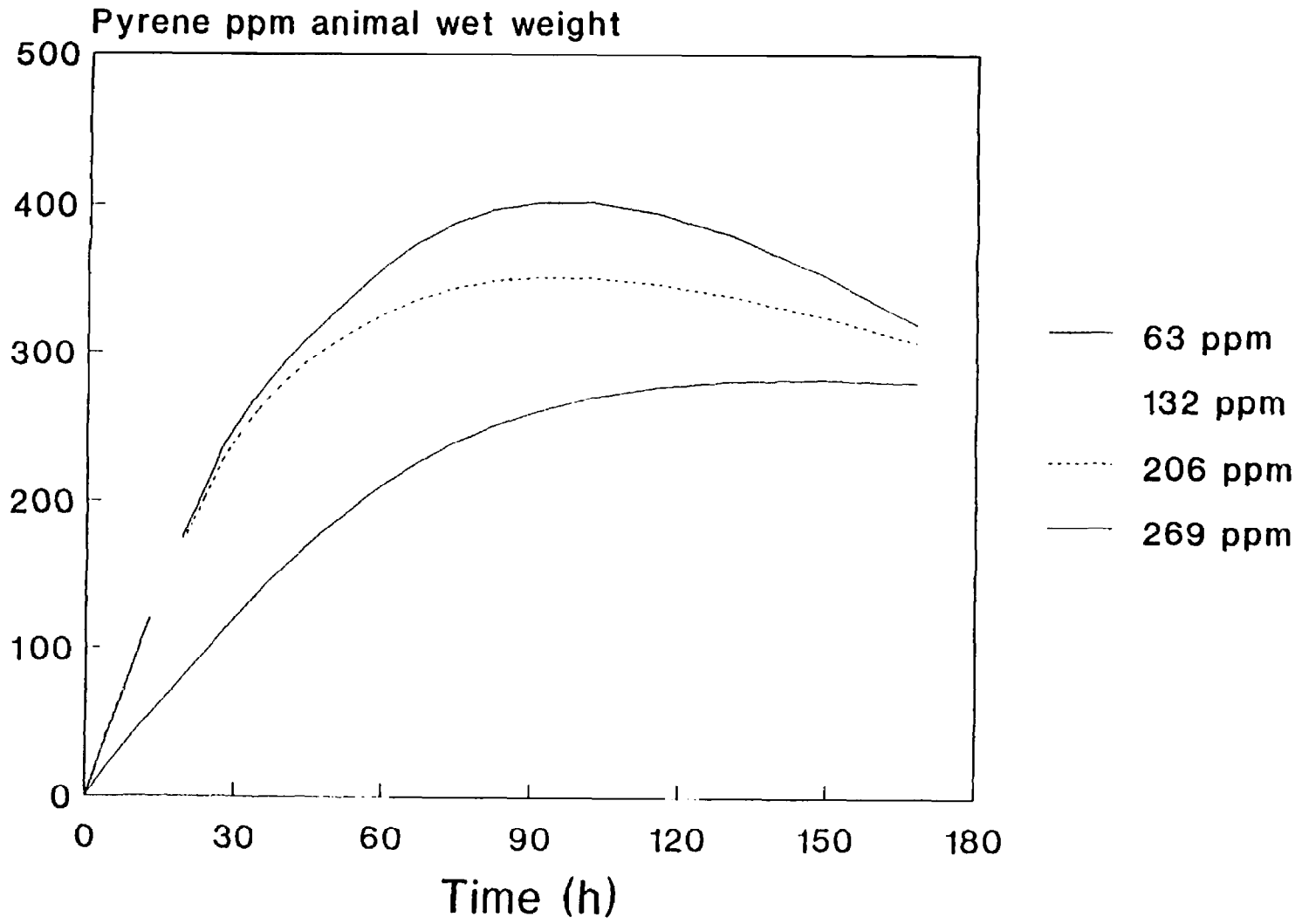
Loss results from loss of material in the intestional tract and elimination of the compound from body tissues.

From kinetic determinations the intestional content of the organism was nondetectable for hexachlorobiphenyl and accounted for 20% of the benzo(a)pyrene body burden after 7 days of exposure. The 20% difference was not statistically significant.

From separate assimilation study only 10.5 to 10.9% of the body burden was from intestional contents for benzo(a)pyrene.

26.8 % of the hexachlorobiphenyl and 31.9% of the benzo(a)pyrene will be lost with 24 h gut purging

Pyrene accumulation by L. variegatus



Development of a Standard Acute Amphipod Protocol

Richard C. Swartz, U.S. EPA Environmental Research Laboratory - Pacific Division

I. Generic Protocol Design

II. Generic Technical Issues

III. Species Specific Modifications/Issues

A.	Rhepoxynius abronius	(Rick Swartz)
B.	Ampelisca abdita	(Michele Redmond)
C.	Eohaustorius estuarius	(Janet Lamberson)
D.	Leptocheirus plumulosus	(Beth McGee)
E.	Lepidactylus dysticus	(Ray Alden)
F.	Canadian Test Species	(Richard Scroggins/Peter Chapman)

IV. Research Needs/Priorities

TEMPERATURE AND SALINITY

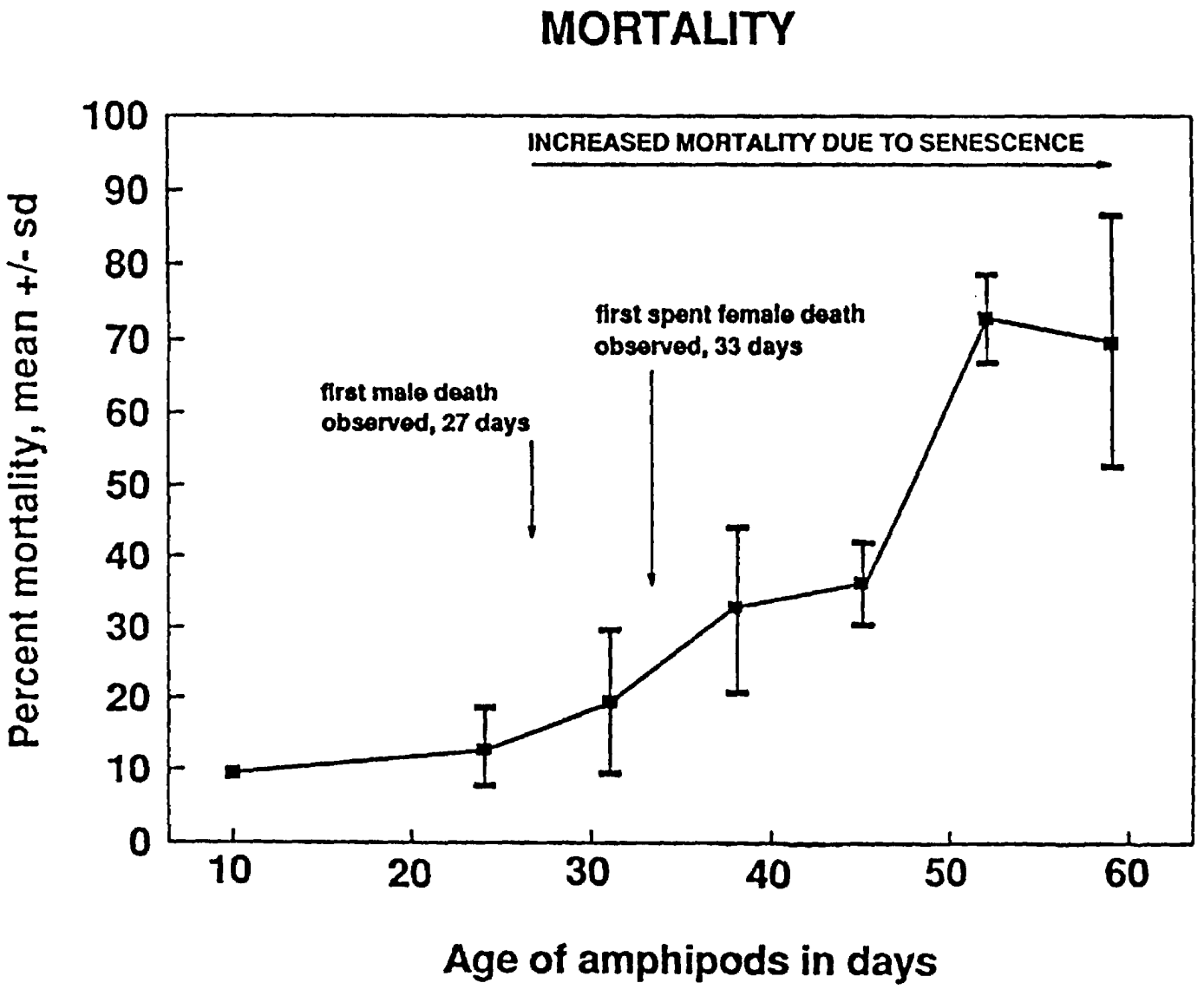
CONDITIONS: start with 30 adults per replicate, static renewal with algal food

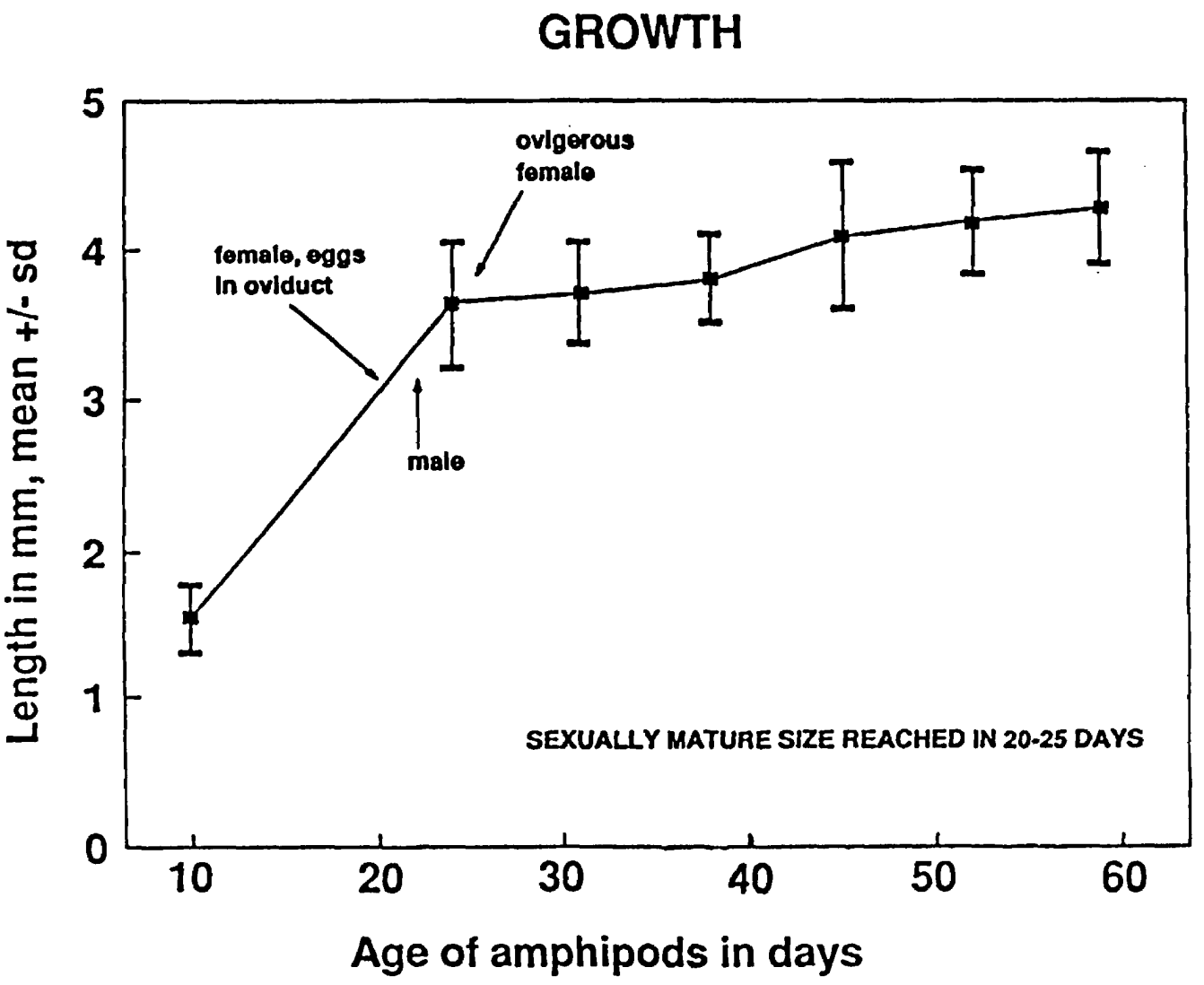
	NUMBER RECOVERED			
	25°C		20°C	
	20ppt	30ppt	20ppt	30ppt
8 weeks	399	520	233	315
9 weeks	121	342	212	490
10 weeks	178	401	275	219

LIFE CYCLE AT 25°C

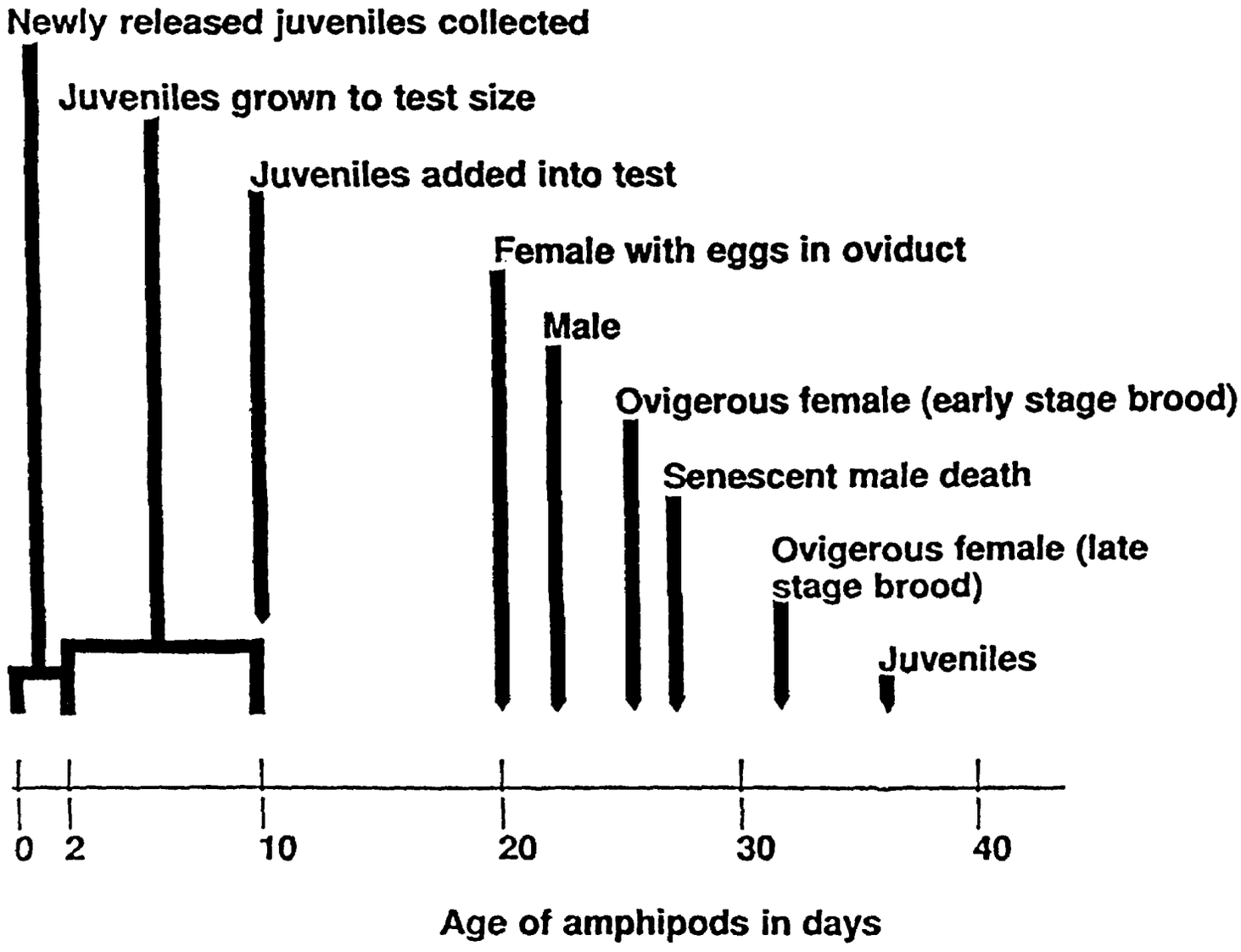
METHODS

- Newly-released juveniles isolated from brooding females in seawater, then held 8-10 days
- 10 juveniles/replicate
- 25°C, 30ppt, 16 hrs light
- Fed the flagellate Pseudoisochrysis paradoxa
- Flow-through system, 1 VR/day (seawater + food)
- 3 replicates sampled/week for 7 weeks


MORTALITY
INCREASED MORTALITY DUE TO SENESCENCE
first spent female death observed, 33 days
first male death observed, 27 days
Percent mortality, mean +/- sd
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
Age of amphipods in days


GROWTH
Length in mm, mean +/- sd
0
1
2
3
4
5
10
20
30
40
50
60
Age of amphipods in days
ovigerous female
female, eggs in oviduct
male
SEXUALLY MATURE SIZE REACHED IN 20-25 DAYS

LIFE CYCLE OBSERVATIONS

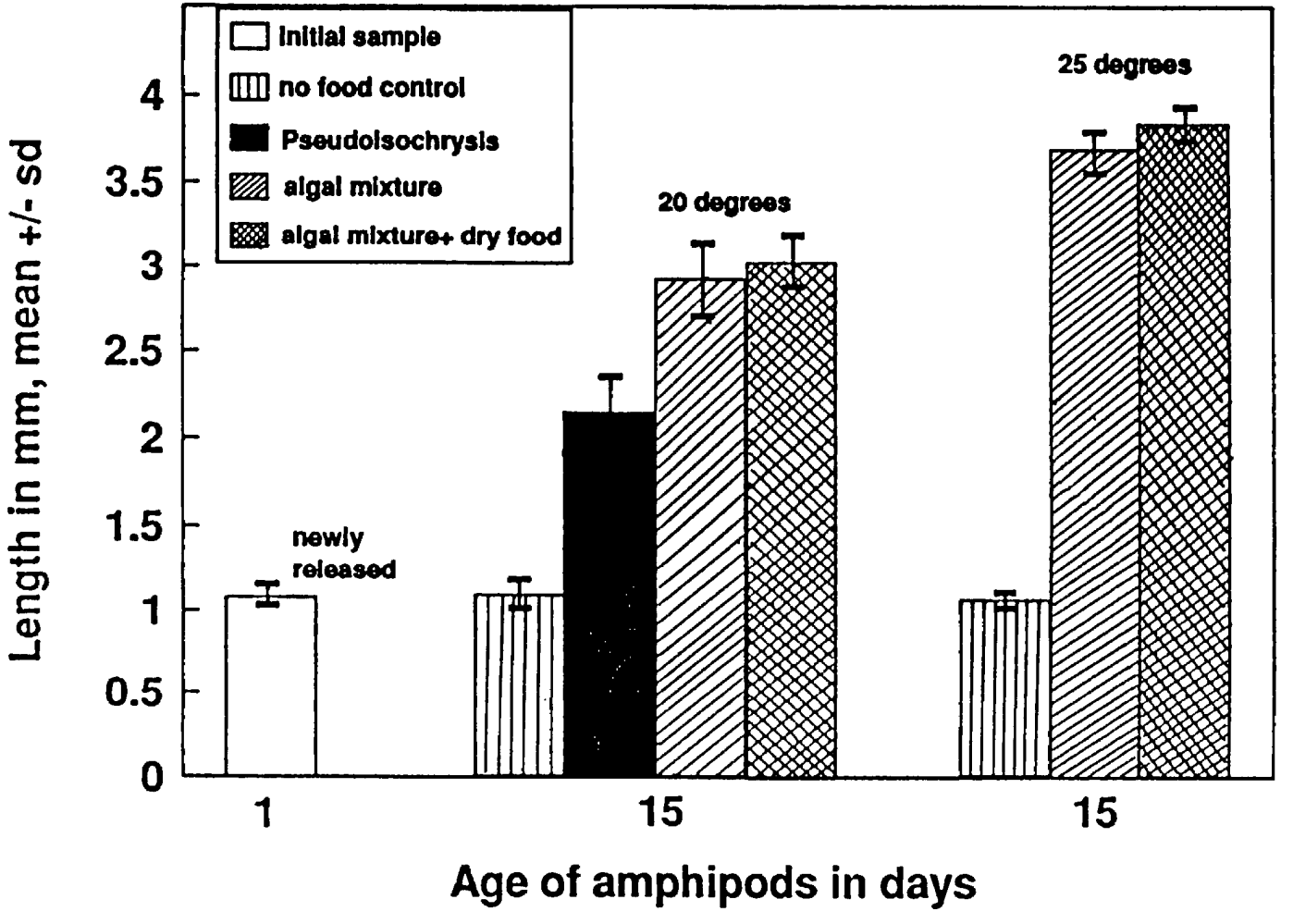


%SURVIVAL AFTER 10 DAYS AT 20°C

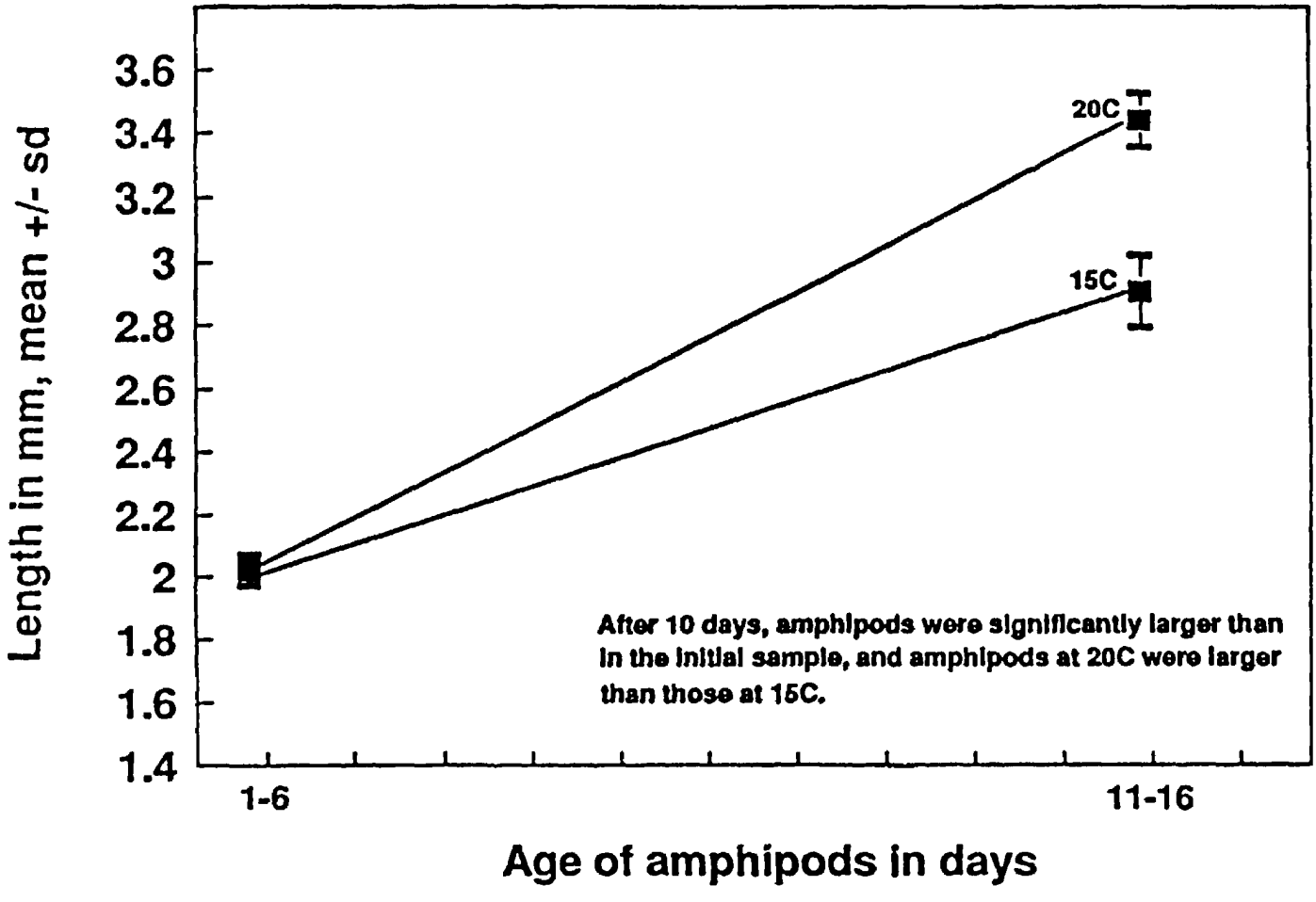
DIFFERENT AMPHIPOD SOURCES AND SEDIMENTS

ANIMAL SOURCE	SEDIMENT	%SURVIVAL
offspring of field-	Long Island	65.0
collected and shipped	Yaquina Bay	78.3
cultures	Long Island	96.7
	Yaquina Bay	98.3

SIGNIFICANT GROWTH DIFFERENCE DETECTED AT 14 DAYS VARYING TEMPERATURE AND NUTRITION



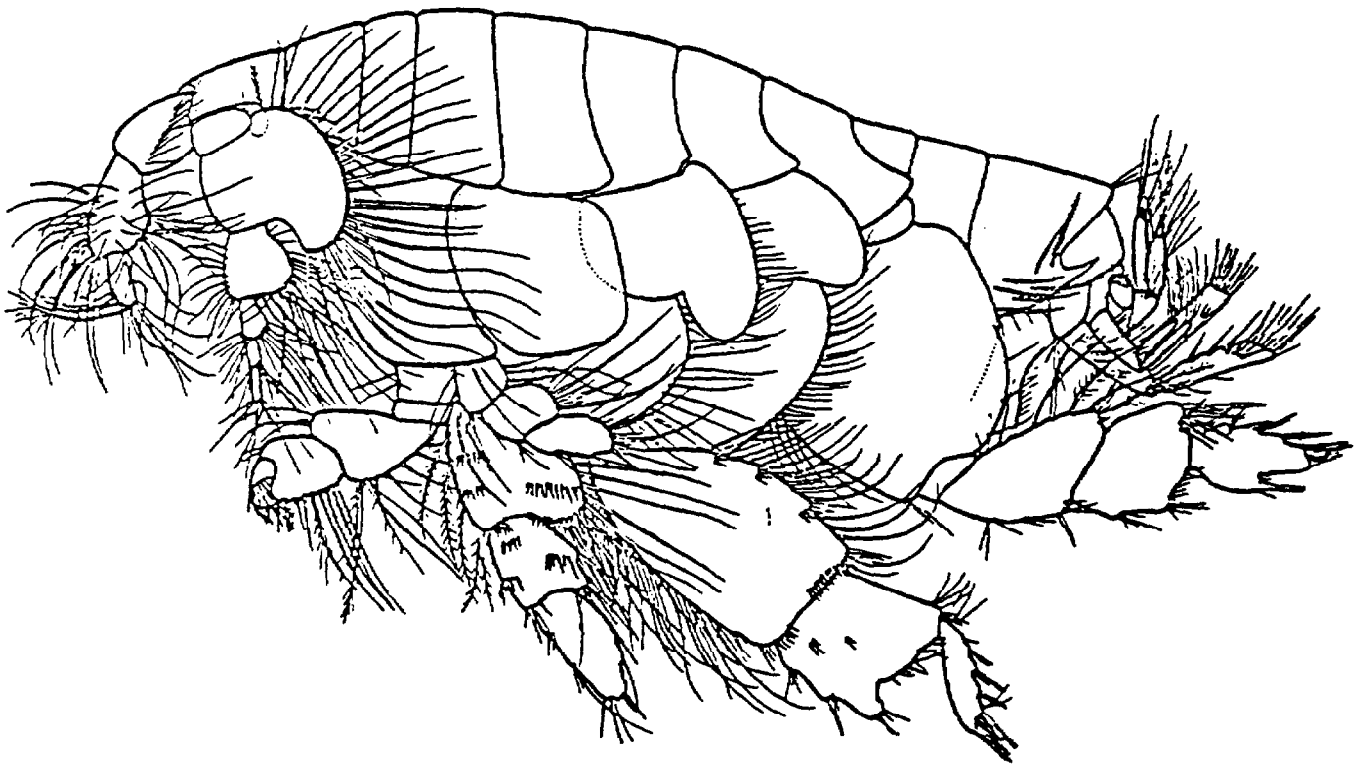
SIGNIFICANT GROWTH DIFFERENCE DETECTED IN 10 DAYS



SUMMARY

- Potential for culture, and chronic and short-term growth tests
- Draft chronic protocol generated
  - Known-age juveniles can be isolated from ovigerous females
  - Start with either newly-released or 10-day old
  - Survival curves can help determine acceptable controls
- Low reproduction and survival in some experiments
  - shipping/handling?
  - need for flow-through >1 vr/day?
  - photoperiod?
  - ????

EOHAUSTORIUS



EOHAUSTORIUS ESTUARIUS (HAUSTORIDAE)

Geographic range:	Central B.C. to central California (other haustorids along Atlantic, Pacific and Gulf coasts)
Habitat:	Free-burrowing sand dweller, upper intertidal to shallow subtidal
Nutrition:	Probable deposit feeder
Life cycle:	Probably annual, not cultured
Source of amphipods:	Field collected, sandy sediment (shovel, sieve, bucket)
Life stage tested:	Large immature to mature, both sexes

ACUTE SEDIMENT TOXICITY TEST CONDITIONS FOR EOHAUSTORIUS ESTUARIUS

Temperature:	15°C (5 to 25°C)
Salinity:	28 ppt (2-35 ppt)
Photoperiod:	Continuous light
Light quality:	Fluorescent lights
Light intensity:	Normal room lights (subdued light for water only tests)
Test chamber sediment depth:	2 cm
Test chamber water volume:	Fill to 950 ml
Number of organisms per test chamber:	20

ACUTE SEDIMENT TOXICITY TEST CONDITIONS FOR EOHAUSTORIUS ESTUARIUS
(continued)

Feeding regime:	No food added during acute test
Aeration:	Air bubbled through a 1-ml diposable glass pipette
Test endpoints:	Emergence, mortality, reburial
Control sediment:	Collection site sediment, 0.5-mm sieved
Grain size:	92% survival in 80% silt-clay 97% survival in sandy sediments
Reference toxicant test:	cadmium chloride, 4-day water only Mean LC50 = 13.05 (4.38-21.72) ** Mean EC50 = 7.07 (1.81-12.33) **

** Numbers in parentheses are reference toxicant warning limits (95% confidence limits = mean +/- 2 standard deviations)

EOHAUSTORIUS ESTUARIUS ACUTE SEDIMENT TOXICITY TEST

ADVANTAGES
- Ease of handling and collection from field
- Salinity tolerance over a broad range
- Grain size tolerance
- Year round availability, can be shipped

LIMITATIONS
- Annual life cycle
  - Cannot culture
  - Cannot use for chronic tests
- Variable response to reference toxicant (cadmium)

RESEARCH NEEDED
- Factors affecting sensitivity to reference toxicant
- Interlaboratory comparison of test method
- Tolerance limits - salinity, temperature, grain size
- Field validation

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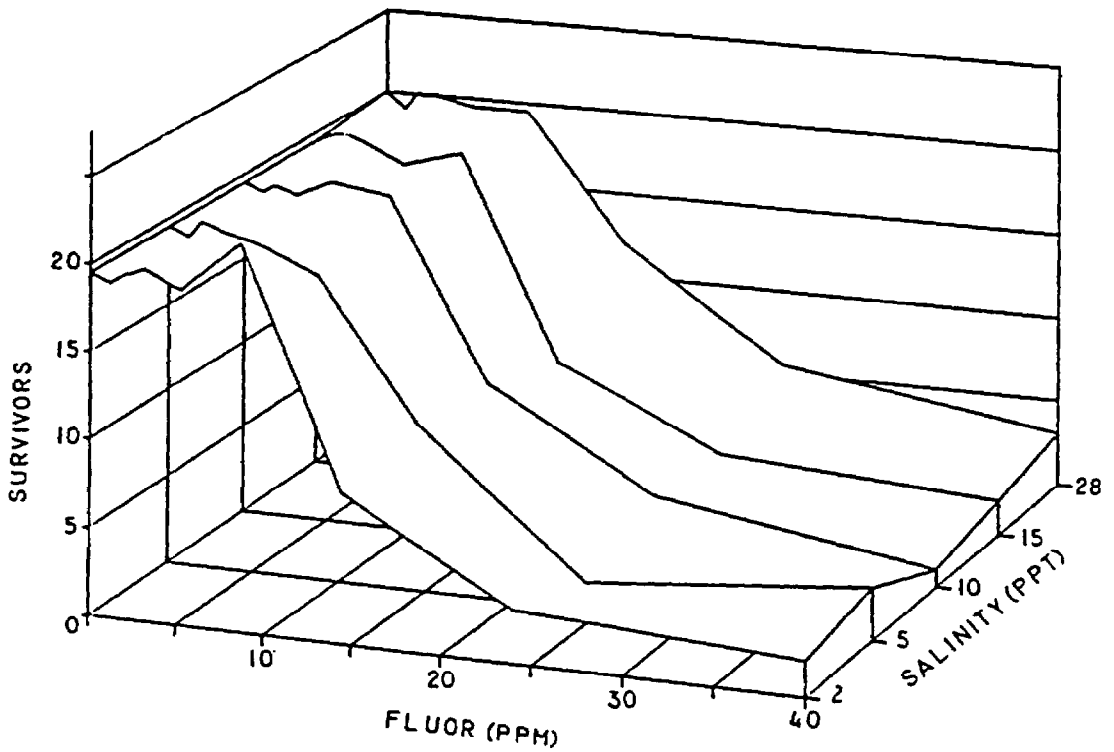
2. Fluoranthene Tolerance: *Eohaustorius estuarius* was slightly more sensitive to fluoranthene than *Hyalella azteca* at 2 ppt salinity, and slightly less sensitive than *Rhepoxynius abronius* at 28 ppt. There was no significant interaction between salinity and fluoranthene tolerance for *E. estuarius*.

Eohaustorius–Salinity–Fluoranthene



Species	Salinity	LC50	95% C.L. Upper	Lower
Hyalella	2 ppt	21.2	18.1	24.8
Eohaustorius	2	13.8	12.3	16.0
Eohaustorius	5	14.0	12.5	15.8
Eohaustorius	10	15.1	13.2	17.0
Eohaustorius	15	13.9	12.2	15.9
Eohaustorius	28	17.5	14.9	20.5
Rhepoxynius	28	6.6	5.9	7.4

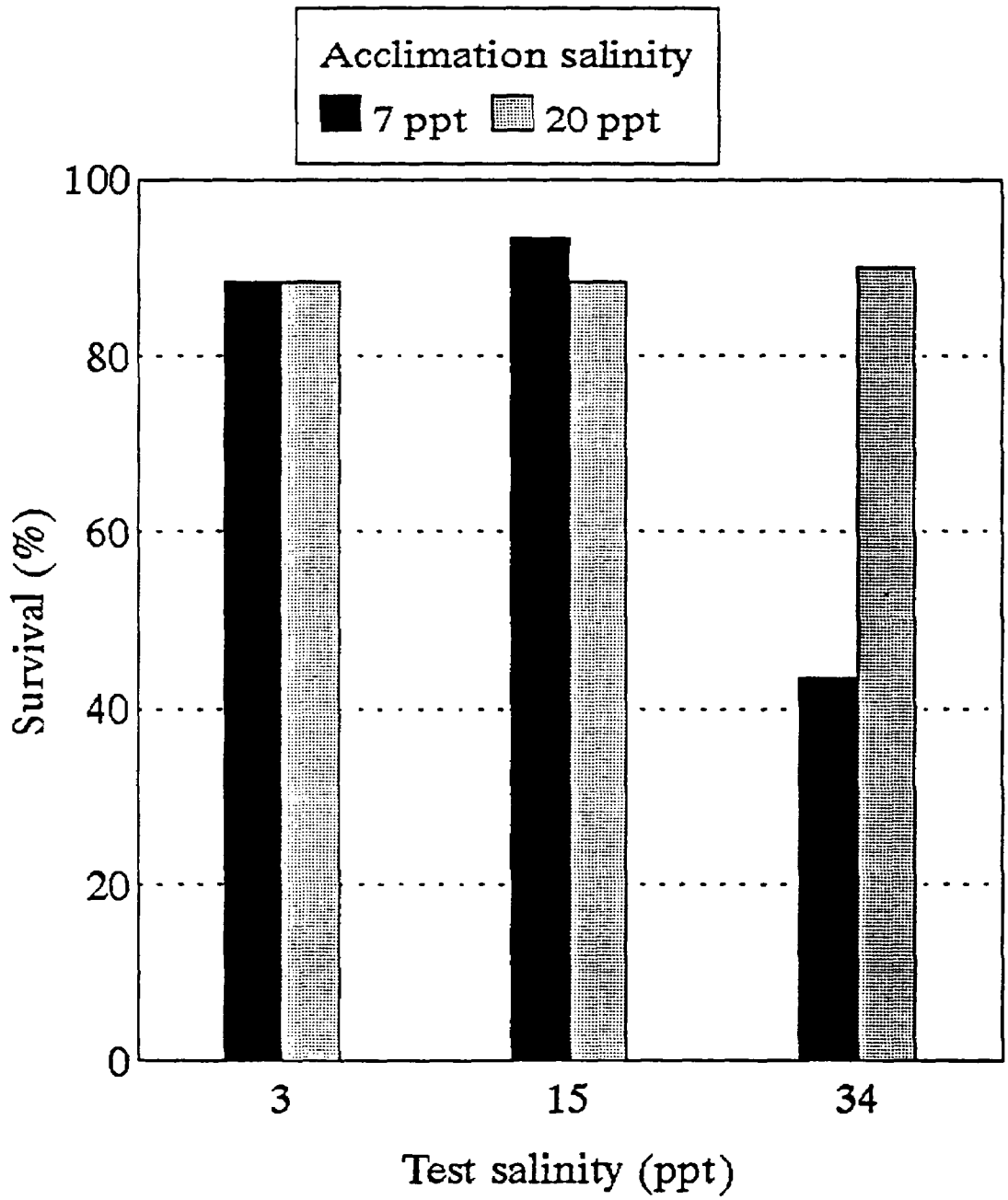
SUMMARY OF 10 D SEDIMENT TOXICITY TEST CONDITIONS
USING Leptocheirus plumulosus

1.	Temperature	20; 25 °C
2.	Salinity	Dependent on objectives of the study
3.	Photoperiod	16:8 light:dark
4.	Volume of test chamber	1 L
5.	Water:sediment ratio	≈ 4:1 (v:v)
6.	Size/age of test organism	3 - 5 mm
7.	No. of organisms/chamber	20
8.	Overlying water	Synthetic sea salts; natural seawater
9.	Negative control	Fine sediment (>85% silt clay); salinity ?
10.	Positive control	96h aqueous $CdCl_2$ @ 20 °C and 20 ‰
11.	Source	Field collected; cultured

Research topics

- Effects of salinity on test results
  - Is acclimation necessary?
  - Will the acclimation salinity influence test sensitivity?
- Sensitivity to common sediment contaminants
  - Expand the chemical database
  - Field validation
  - Interspecies comparisons
- Sensitivity differences among sources of amphipods
  - Laboratory reared versus field collected

Effect of acclimation salinity on survival of juvenile *Leptocheirus* in 10 d exposures



Laboratory Reared Versus Field Collected Amphipods in Sediment Toxicity Tests

ADVANTAGES

Laboratory reared	Field collected
Year round availability	Subsample of a "natural" population
Geographic availability	Easy to obtain large numbers with minimal effort and cost
Reared under known, controlled conditions	

DISADVANTAGES

$ Genetic effects (e.g., inbreeding, selection)	Limited availability (seasonal, geographic)
$ Influence of culture condition on test sensitivity	$ Seasonal &/or geographic variation in sensitivity
Cost (time & money)	

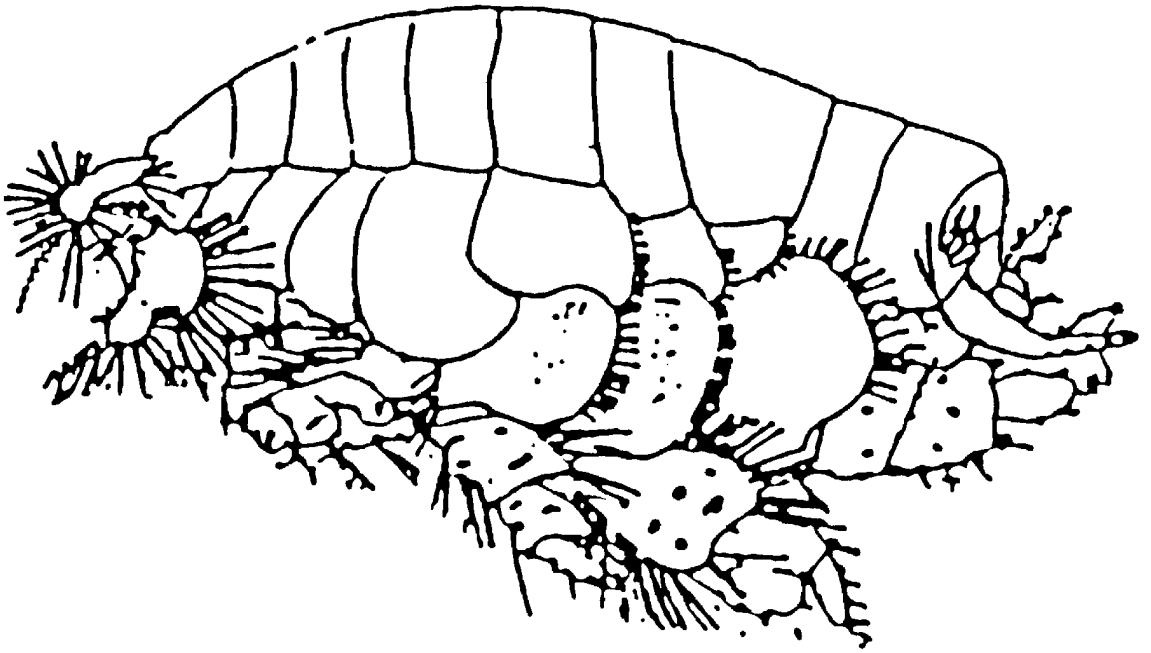
$ Potential research topics

Lepidactylus dytiscus Distribution and Ecology:

Lepidactylus dytiscus is broadly distributed throughout the upper Chesapeake Bay and its tributaries to Florida. It is an intertidal species found 1-2 meters above the low tide mark in moist coarse sand in summer to approximately 1-3 meters below low tide in winter months. *L. dytiscus* burrows freely in coarse sand throughout the year generally burrowing to 4 cm in summer and 6-7 cm in winter. Densities can be 1200-1500 individuals/M^2. Average densities are approximately 150-200/M^2 where *L. dytiscus* occurs.

Bousfield (1970) reports feeding in *L. dytiscus* is by suspension, although it may supplementarily deposit feed. Lab held animals are fed small amounts of *Artemia salina* and algae.

Reproduction generally occurs spring through fall with large females overwintering. Bimodal reproduction appears to be the rule with early spring and late summer recruitment appearing to be greatest.



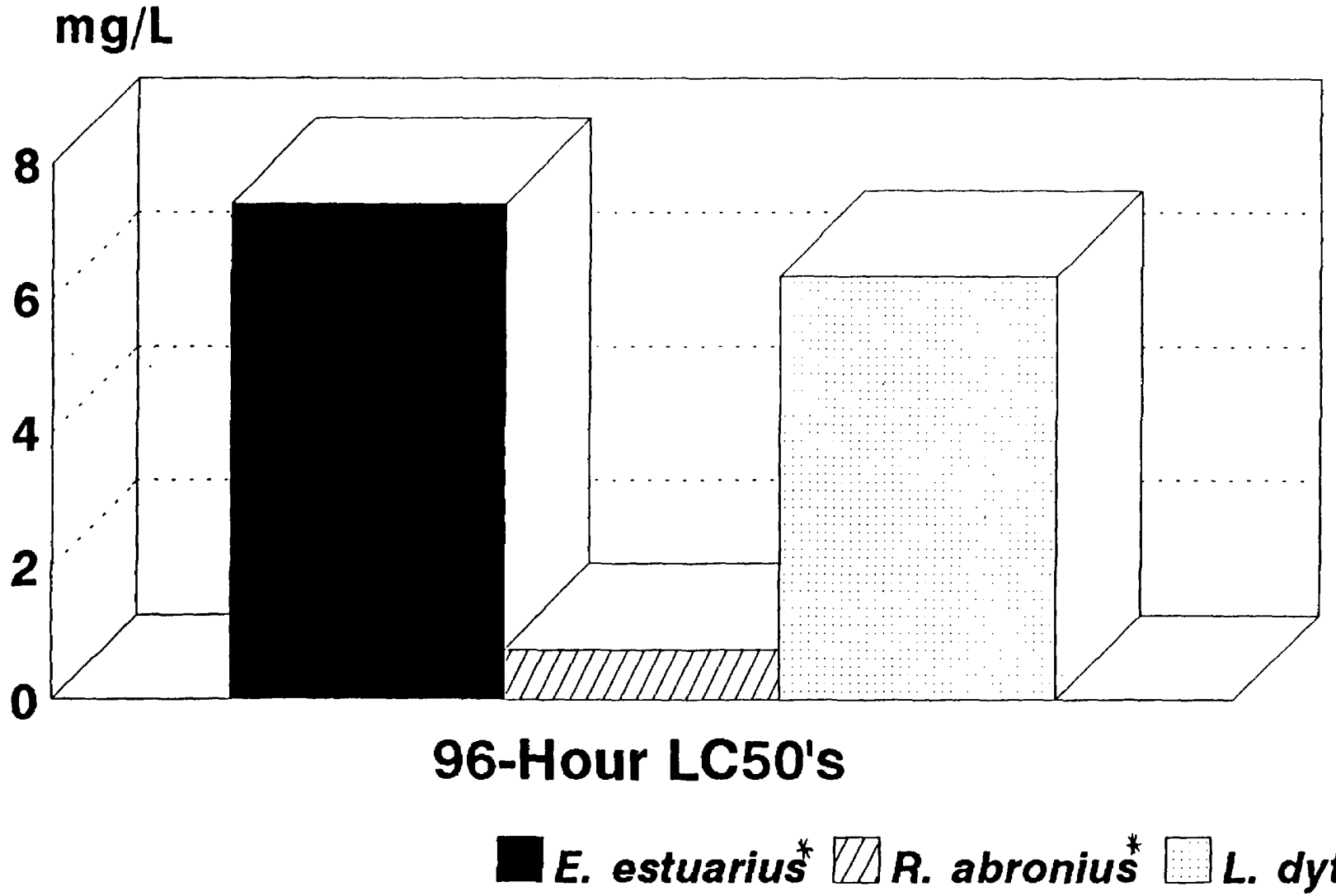
From Grant and Lazo-Wasem (1982)

CULTURE CONDITION FOR LEPIDACTYLUS DYTISCUS AT AMRL

R. Alder

CULTURE CONDITION	CONDITION USED BY LABORATORY
1. Test type	Static non-renewal
2. Temperature	20 + 2°C
3. Light quality	ambient laboratory
4. Light intensity	ambient laboratory
5. Photoperiod	16:8 L:D
6. Culture chamber size	20 gallons
7. Culture water volume	18 gallons
8. Restart duration	1 yr
9. Renewal of culture water	50% weekly
10. Removal of offspring (frequency)	2/yr
11. Age of restart organisms	Various
12. No. of organisms/culture chamber	1200
13. No. of culture tanks	3
14. Feeding regime	artemia (twice/week)
15. Substrate used	native coarse sand
16. Chamber cleaning	occasional stirring and siphoning
17. Aeration	moderate w/filtration
18. Culture water	DI w/ artificial sea salts

COMPARISON OF AMPHIPOD SENSITIVITIES
(Cadmium, Water Only Exposure, 28ppt)



*From DeWitt et al., 1989

REFERENCE TOXICANT DATA:

R. Alden

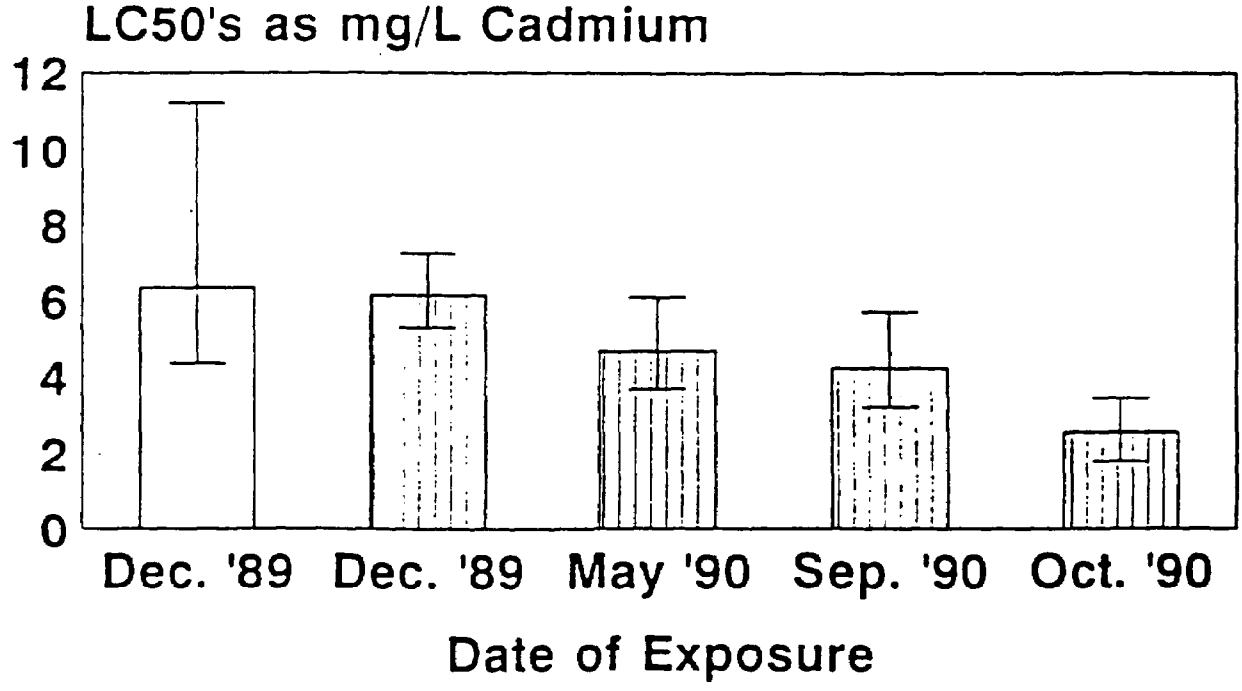
CADMIUM CHLORIDE TEST:

At 28 ppt the measured LC50 was 6.33 mg/L Cd (95% CI 11.22-4.35) with a control survival of 97% (EPA Probit Analysis, Version 1.4).

At 20 ppt the measured LC50 was 6.13 mg/L Cd (95% CI 7.25 and 5.3), with a control survival of 97% (EPA Probit Analysis, Version 1.4).

Results of reference toxicant tests conducted at intervals throughout the year showed sensitivities in the range of 6.3 to 2.5 mg/L Cd, with a trend toward increasing sensitivity in the early fall population.

CADMIUM CHLORIDE REFERENCE TOXICANT TEST
(96-Hour, Water Only)



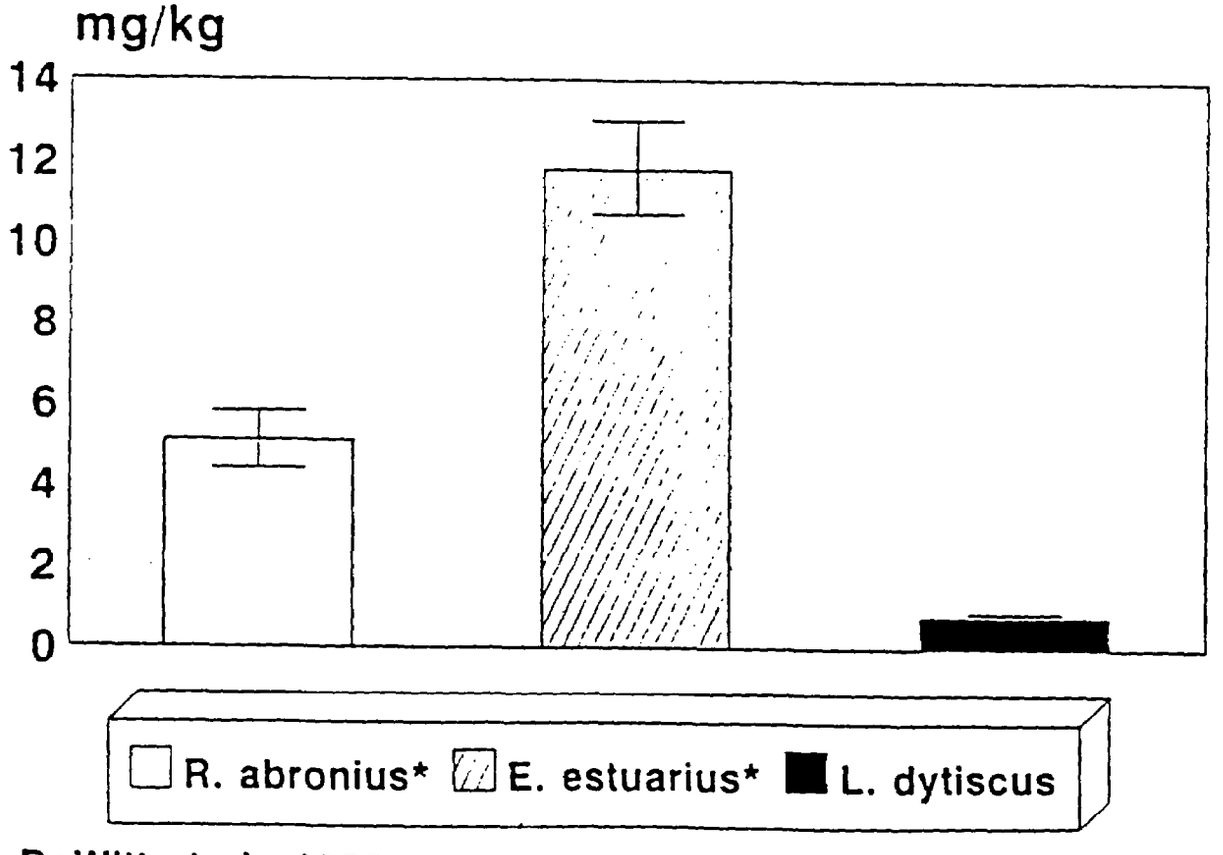
REFERENCE TOXICANT DATA:

FLUORANTIIENE TEST:

An average survival of 94% was found in both the sediment and the acetone controls.

Calculations using the Moving Average Angle Method indicated a nominal LC50 of 1.44 mg/kg (95% confidence intervals 1.33 and 1.55 mg/kg) and a measured LC50 value of 0.793 mg/kg (95% confidence intervals 0.698 and 0.887 mg/kg) fluoranthene.

COMPARISON OF AMPHIPOD SENSITIVITIES
(10-Day Fluoranthene Exposure, 28ppt)



*From DeWitt et al., 1989

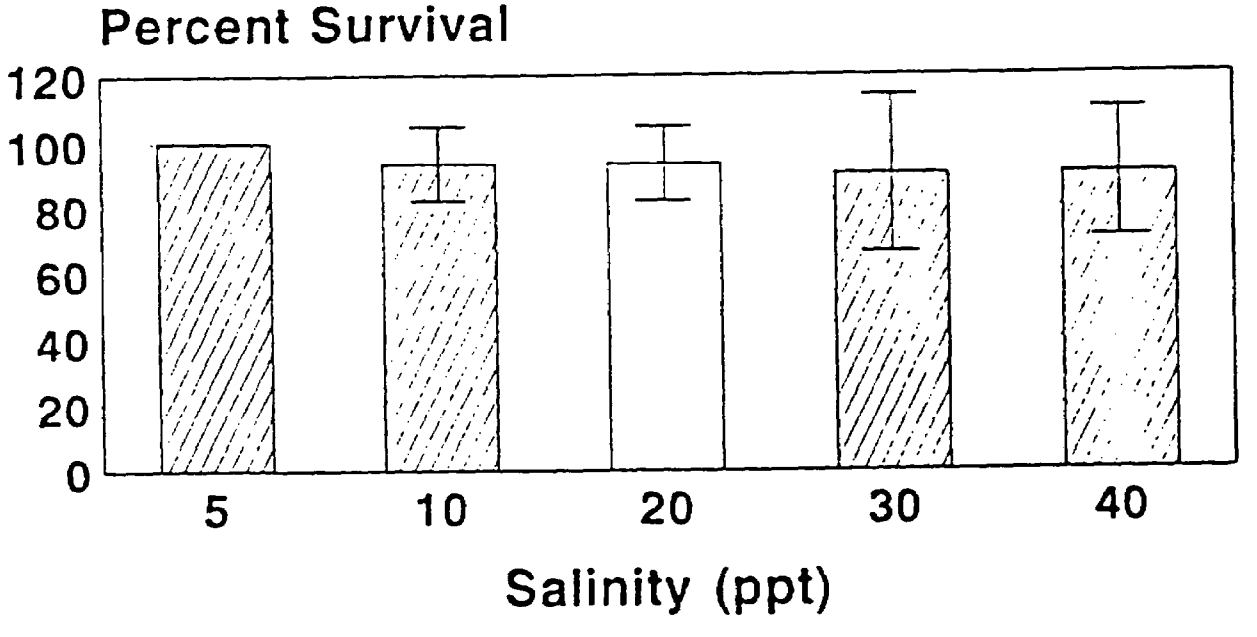
SALINITY TEST:

Statistical analysis using anova and regressions showed no significant difference in survival of amphipods tested in salinities from 5 to 40 ppt.

Average survival in the controls was 93.3%.

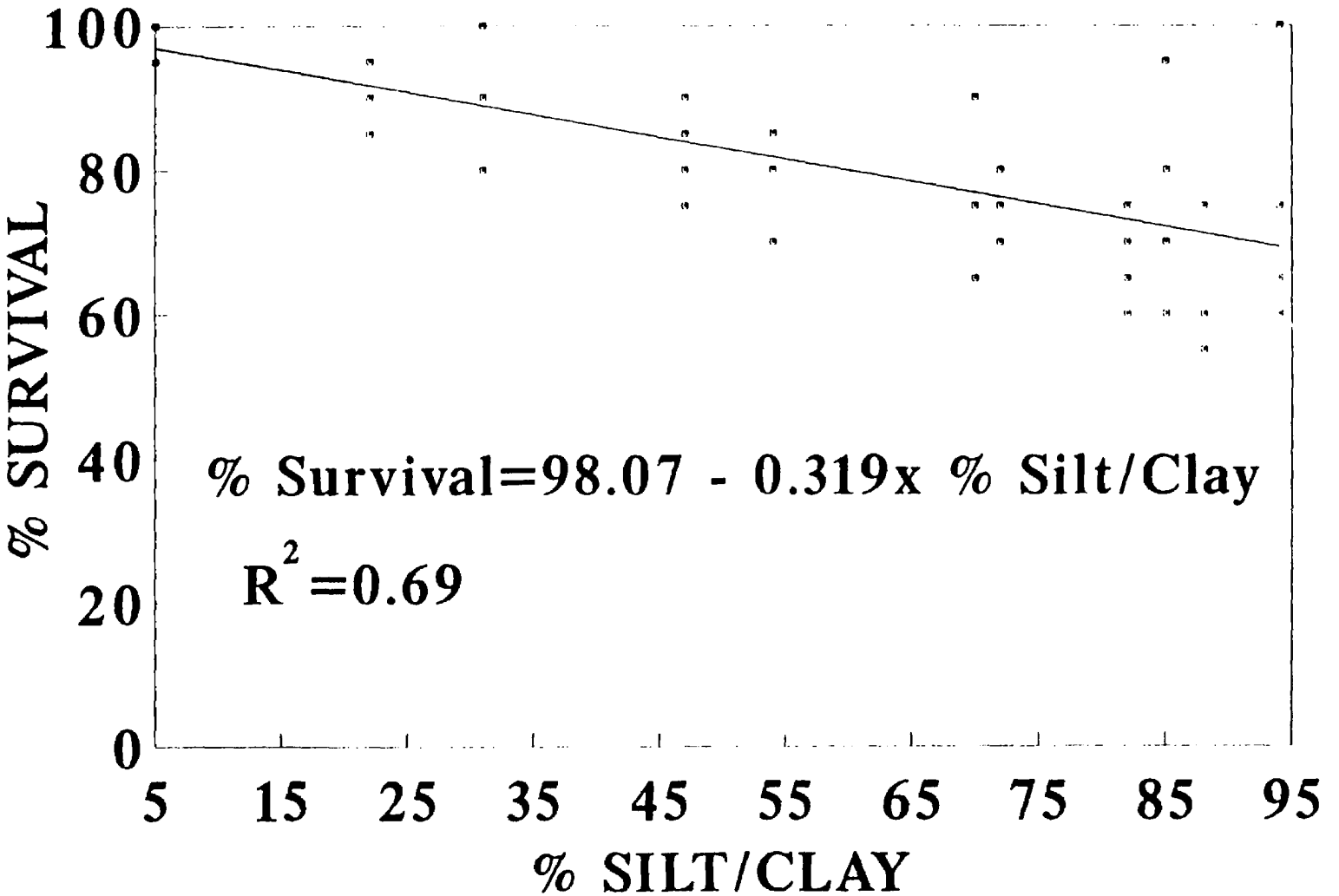
Test survival averaged over all test groups was 93.3% over the 14 day test period, with no less than 90% survival in any group.

SALINITY TOLERANCE
(10-Day Exposure)

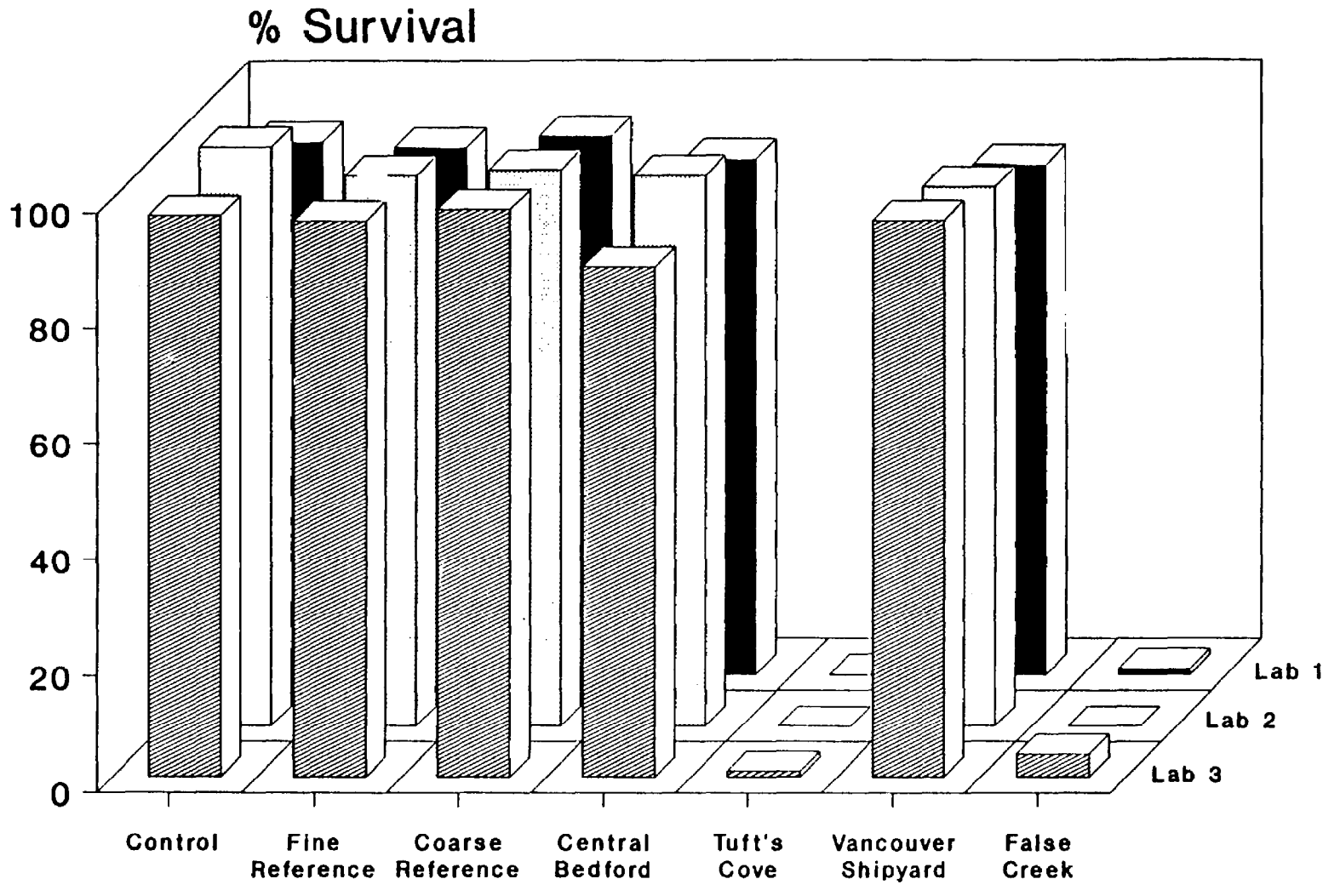


NOTE: 95% Confidence Limits Shown

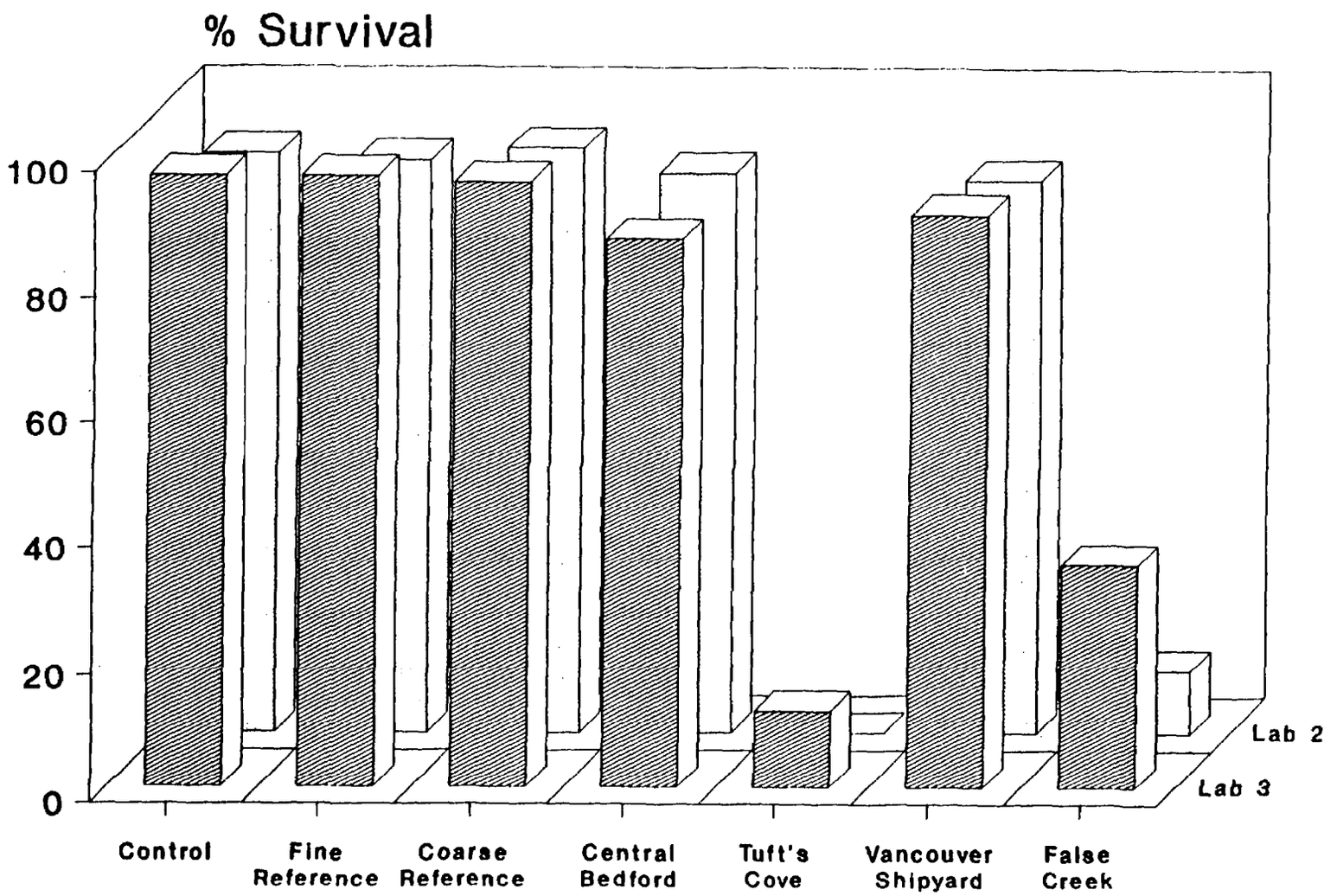
Survival of L. dytiscus vs. % Silt/Clay



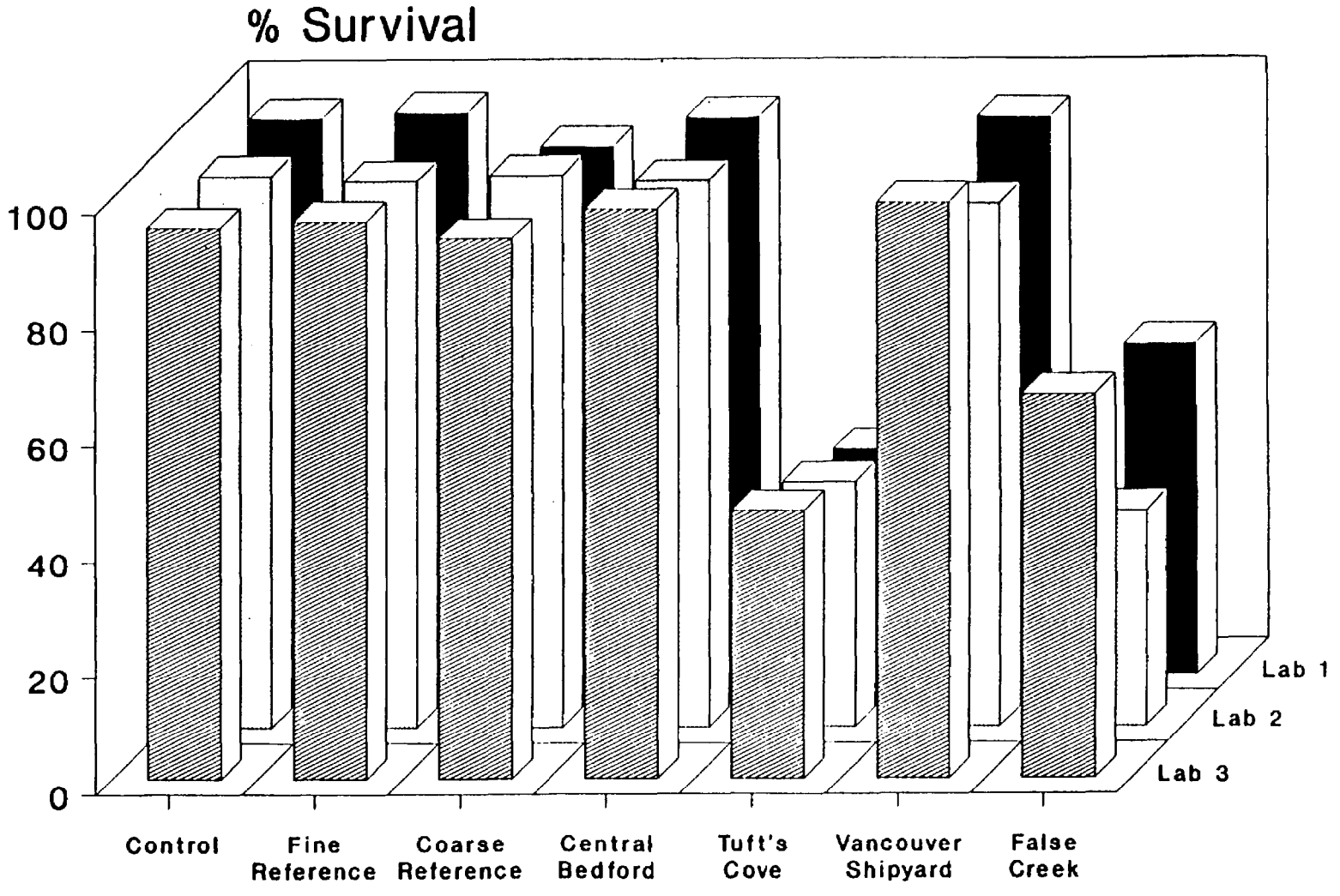
Rhepoxynius abronius



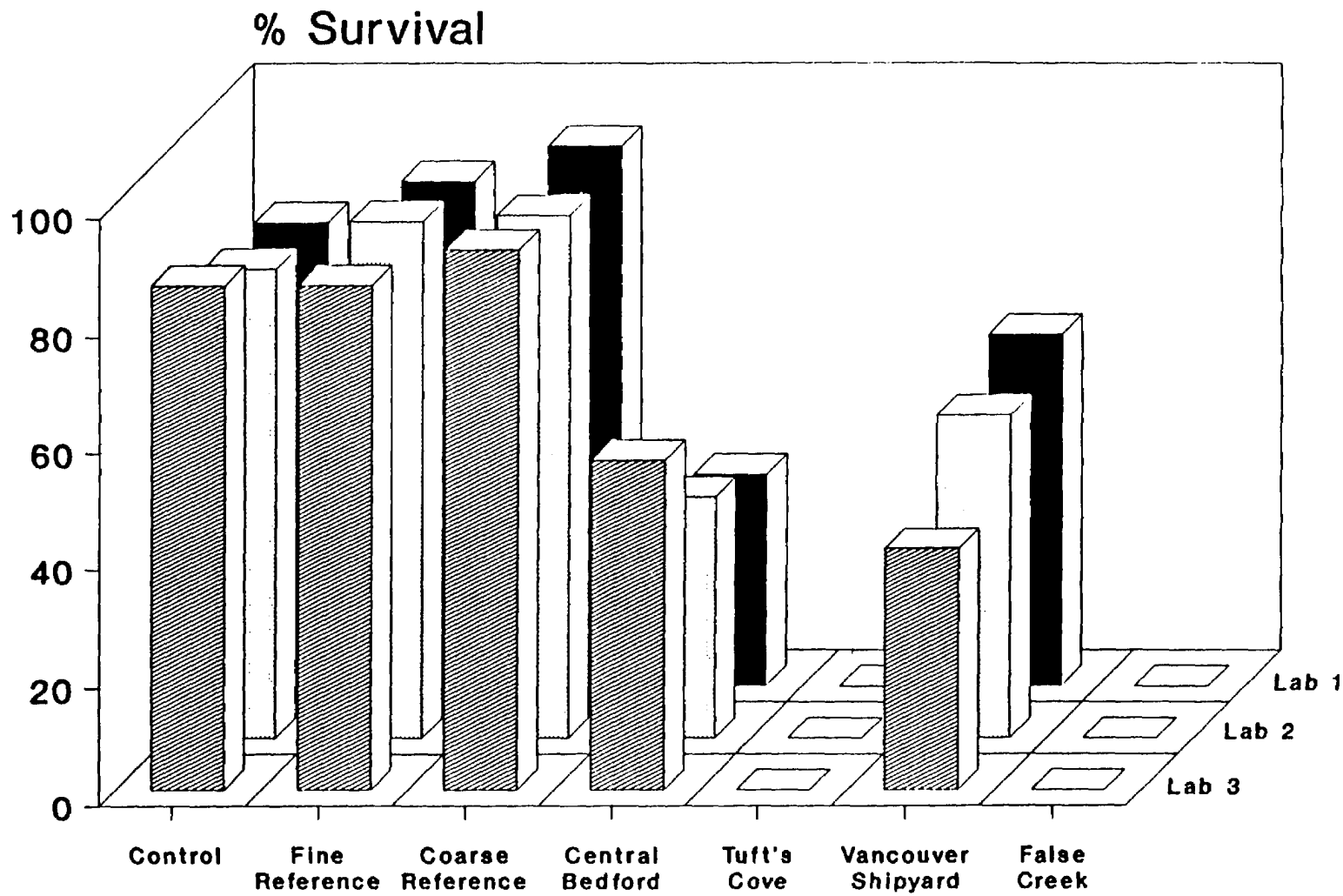
Eohaustorius estuarius



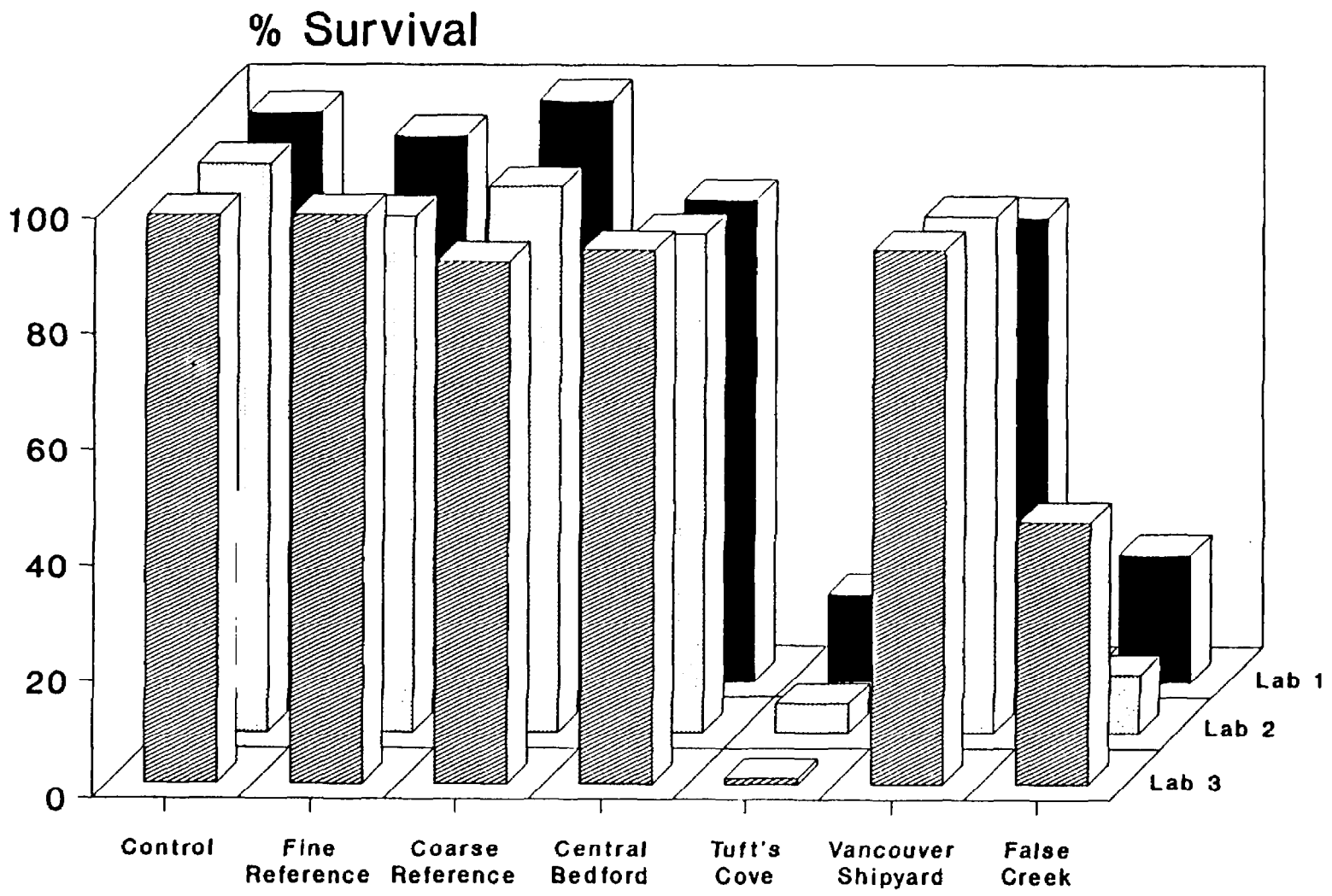
Corophium volutator



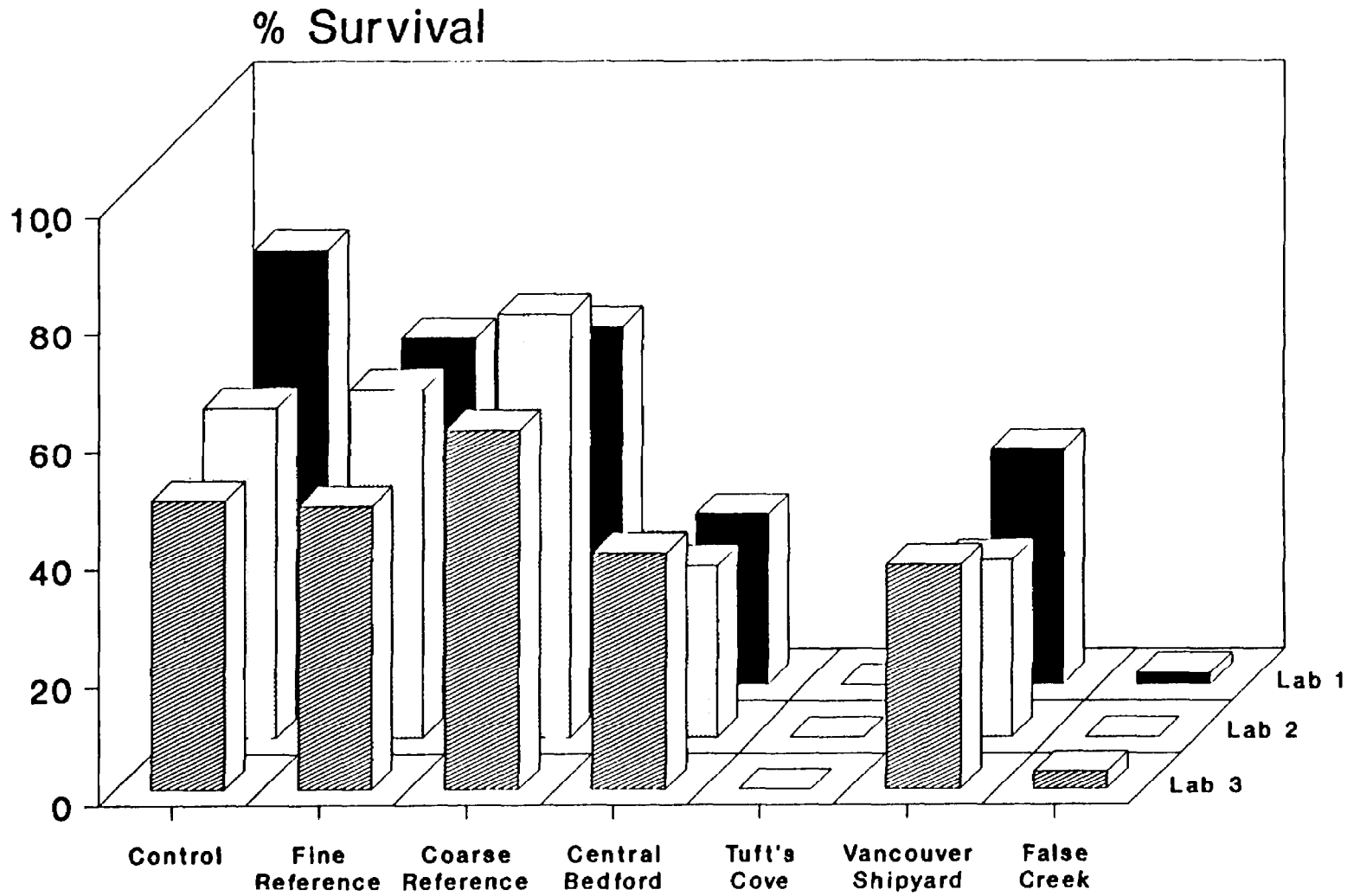
Eohaustorius washingtonianus



Foxiphalus xiximeus



Amphiporeia virginiana



Development of a Standard Chronic Amphipod Protocol

John Scott, SAIC - Narragansett, RI
with contributions from:
Ted DeWitt, AScI - Newport, OR
Michelle Redmond, AScI - Newport, OR
Chris Schlekat, Maryland Department of the Environment - Baltimore, MD

I. Introduction (Scott)

A. Historical background
B. Applications of chronic procedures
C. Minimum requirements of the methods

II. Chronic Procedures Using Leptocheirus plumulosus (Dewitt)

A. Preliminary Leptocheirus plumulosus chronic method

1. Species selection
   a. Culturability
   b. Contaminant sensitivity
   c. Handling

2. Culture methods
   a. Physical requirements
      i. Tubs
      ii. Salinity
      iii. Temperature
      iv. Light
      v. Sediment

3. Test animals
   a. Life stage
   b. Number per replicate
   c. Feeding
   d. Handling and recovery
   e. Responses
      i. Mortality
      ii. Growth
      iii. Reproduction
      iv. Others
   f. Tolerance limits
      i. Grain size
      ii. Salinity
      iii. Organic enrichment
      iv. Seasonality
   g. Special requirements
      i. Quarantine laws and practices

4. Exposure logistics
    a. Chamber characteristics
    b. Exposure duration
    c. Sediment depth and volume
    d. Water change
    e. Aeration
    f. Temperature
    g. Salinity
    h. Dissolved oxygen
    i. Light
        i. Photoperiod
        ii. Intensity and quality
    j. Other environmental variables

5. Experimental design
    a. Treatments
    b. Replication
    c. Statistics
        i. Analytical methods
        ii. Variability and power
    d. Controls
        i. QA/QA
            - Performance (health)
            - Reference toxicant
        ii. Experimental
            - Carrier (spiked sediment)
            - Site (dilution series)
            - Environmental (grain size, TOC, salinity, temperature)
    e. QA/QC
        i. Environmental conditions
        ii. Response criteria (mean, variability)
        iii. Controls

B. Research findings

1. Sensitivity to sediment variables
    a. Mortality and growth

2. Sensitivity to phenanthrene-spiked sediment
    a. Relative sensitivity of mortality, growth, and fertility

3. Sensitivity to contaminated sediment dilution series
    a. Relative sensitivity of mortality, growth, and fertility
4. Nutrition affects growth

C. Comparison of EPA and Maryland Department of Environment methods

D. Research needs

1. Influence of nutrition on toxicological sensitivity

2. Influence of other variables on toxicological sensitivity
   a. Temperature
   b. Salinity
   c. Sediment grain size
   d. Other variables

3. QA/QC issues
   a. Development of reference toxicant method for growth and fertility
   b. Environmental conditions during exposure

4. Relative sensitivity of cultured and field-collected animals

5. Simplification of culture and feeding methods

6. Relative sensitivity to other species (acute and chronic)

7. Experimental design optimization

8. Development of toxicological database (chemical diversity)

9. Inter-laboratory comparison

10. Field validation

II. Chronic Procedures Using Leptocheirus plumulosus (Schlekat)

A. Characteristics of the test organism
B. Selection of the test endpoints
C. Elements of the procedure
D. Representative results
E. Research needs

III. Chronic Procedures Using Ampelisca abdita (Redmond and Scott)

A. Characteristics of the test organism
B. Selection of the test endpoints
C. Elements of the procedure
D. Representative results
E. Research needs

IV. Discussion (Workgroup)

A. Input from workgroup these procedures
B. Experience with other species

V. Summary and Recommendations (Scott)

CHRONIC TEST METHODS

LEPTOCHEIRUS

STATUS:
- PRELIMINARY METHOD
  28-DAY SURVIVAL, GROWTH
  REPRO
- MULTIPLE LABORATORY USE
  MINOR DIFFERENCES
- CULTURE/FIELD COLLECTED

ISSUES:
NUTRITION-DIET QUANT/QUAL
INTER-INTRA LAB VARIABILITY
FIELD VALIDATION
ROLE OF SEDIMENT NUTRITION QUAL
REF TOX FOR CHRONIC ENDPOINTS

GOAL:
DRAFT STANDARD 9/93 → FALL ASTM
GUIDE?

AMPELISCA

STATUS:
PRELIMINARY/SUBLETHAL METHOD
20-DAY - GROWTH
LIFE CYCLE 30-40 DAY-DEVELOPING
CULTURE METHODS - DEVELOPING

ISSUES:
TEST DESIGN FOR REPRO ENDPTS
OTHER SAME AS L.P.

GOAL:
DRAFT STANDARD SUBLETHAL -
GUIDE? CHRONIC?

9/93 → FALL ASTM

TOXICITY TEST INTERPRETIVE GUIDANCE

STATISTICAL SIGNIFICANCE

DETECTABLE SIGNIFICANCE

BIOLOGICAL SIGNIFICANCE

INTERPRETATIVE GUIDANCE

DETECTABLE SIGNIFICANCE

Statistical significance incorporates within

test variability among replicates.

.....but what about test performance

variability???

INTERPRETATIVE GUIDANCE

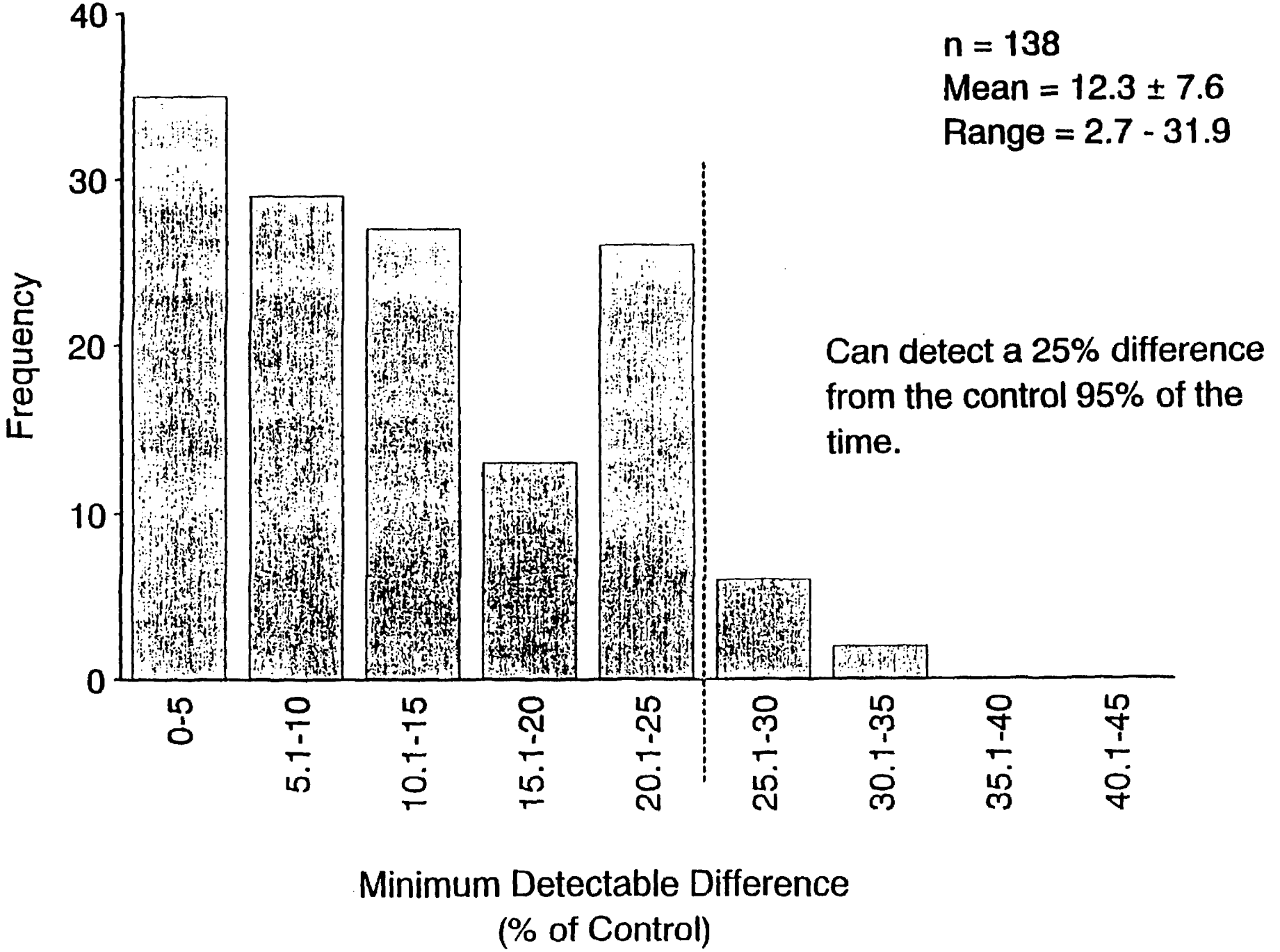
DETECTABLE SIGNIFICANCE

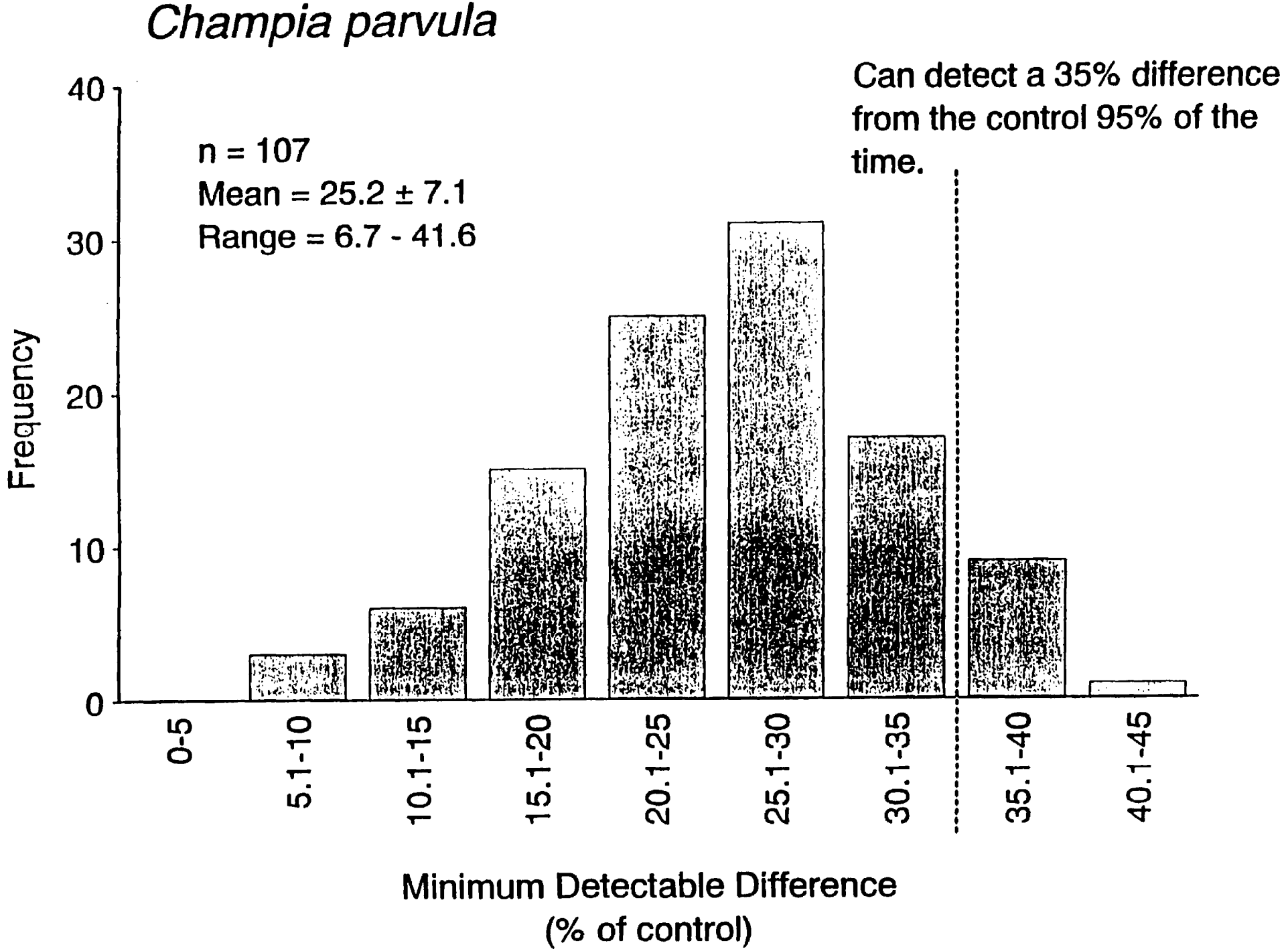
CALCULATION OF THE LEAST SIGNIFICANT DIFFERENCE (LSD)

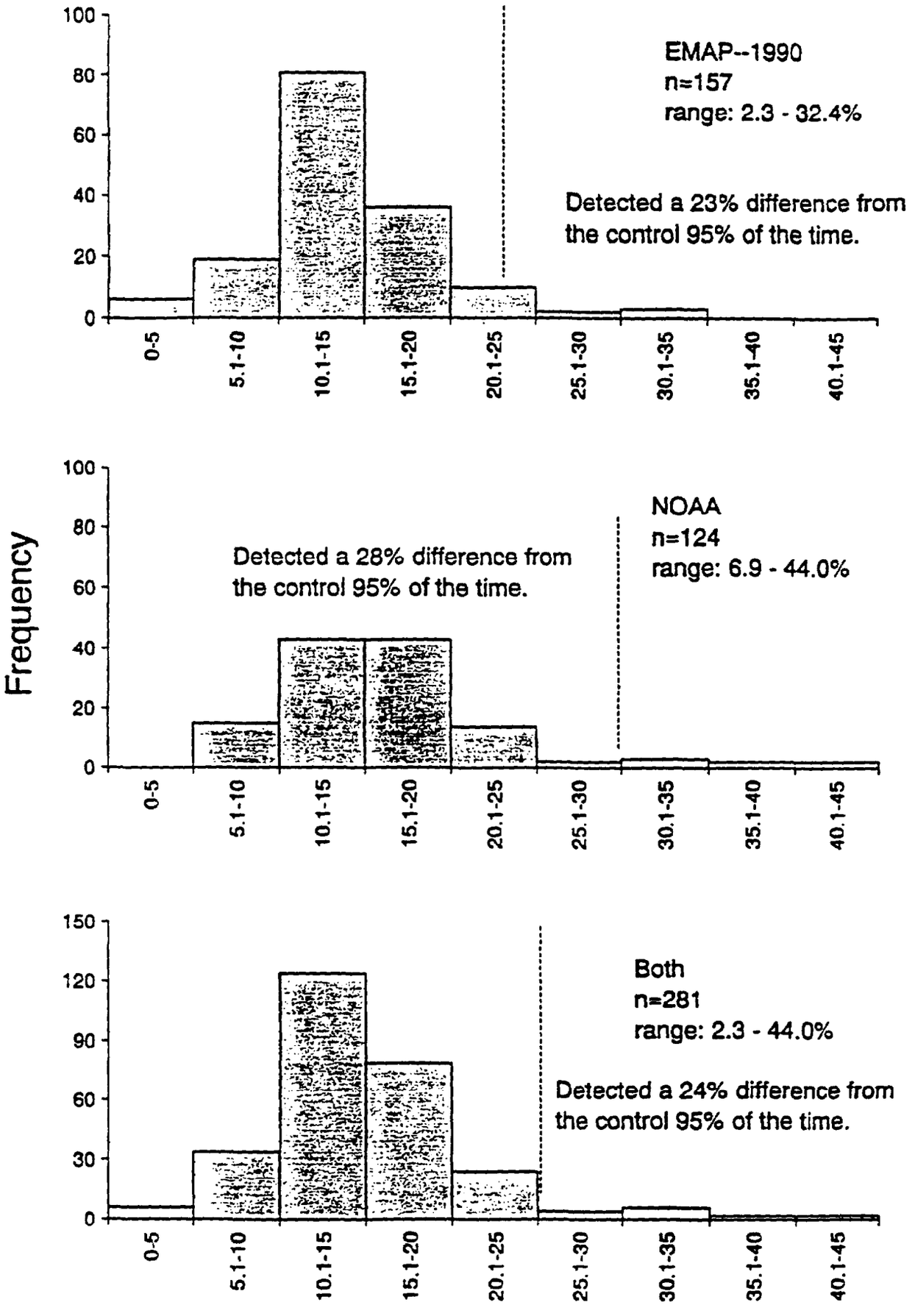
- o Conduct one-way *t*-test
- o Assume unequal variances
- o Generate a *t* value
- o Conduct ANOVA to generate MSE

$$LSD = t_{df(i),0.05}\ SqRt\ ((1/N_c + 1/N_i) * MSE)$$

Arbacia punctulata




Champia parvula
Can detect a 35% difference from the control 95% of the time.
n = 107
Mean = 25.2 ± 7.1
Range = 6.7 - 41.6
Frequency
40
30
20
10
0
0-5
5.1-10
10.1-15
15.1-20
20.1-25
25.1-30
30.1-35
35.1-40
40.1-45
Minimum Detectable Difference
(% of control)


EMAP--1990
n=157
range: 2.3 - 32.4%
Detected a 23% difference from the control 95% of the time.
NOAA
n=124
range: 6.9 - 44.0%
Detected a 28% difference from the control 95% of the time.
Both
n=281
range: 2.3 - 44.0%
Detected a 24% difference from the control 95% of the time.
Frequency
0-5
5.1-10
10.1-15
15.1-20
20.1-25
25.1-30
30.1-35
35.1-40
40.1-45
Least Significant Difference (% of Control Survival)

INTERPRETATIVE GUIDANCE

BIOLOGICAL SIGNIFICANCE

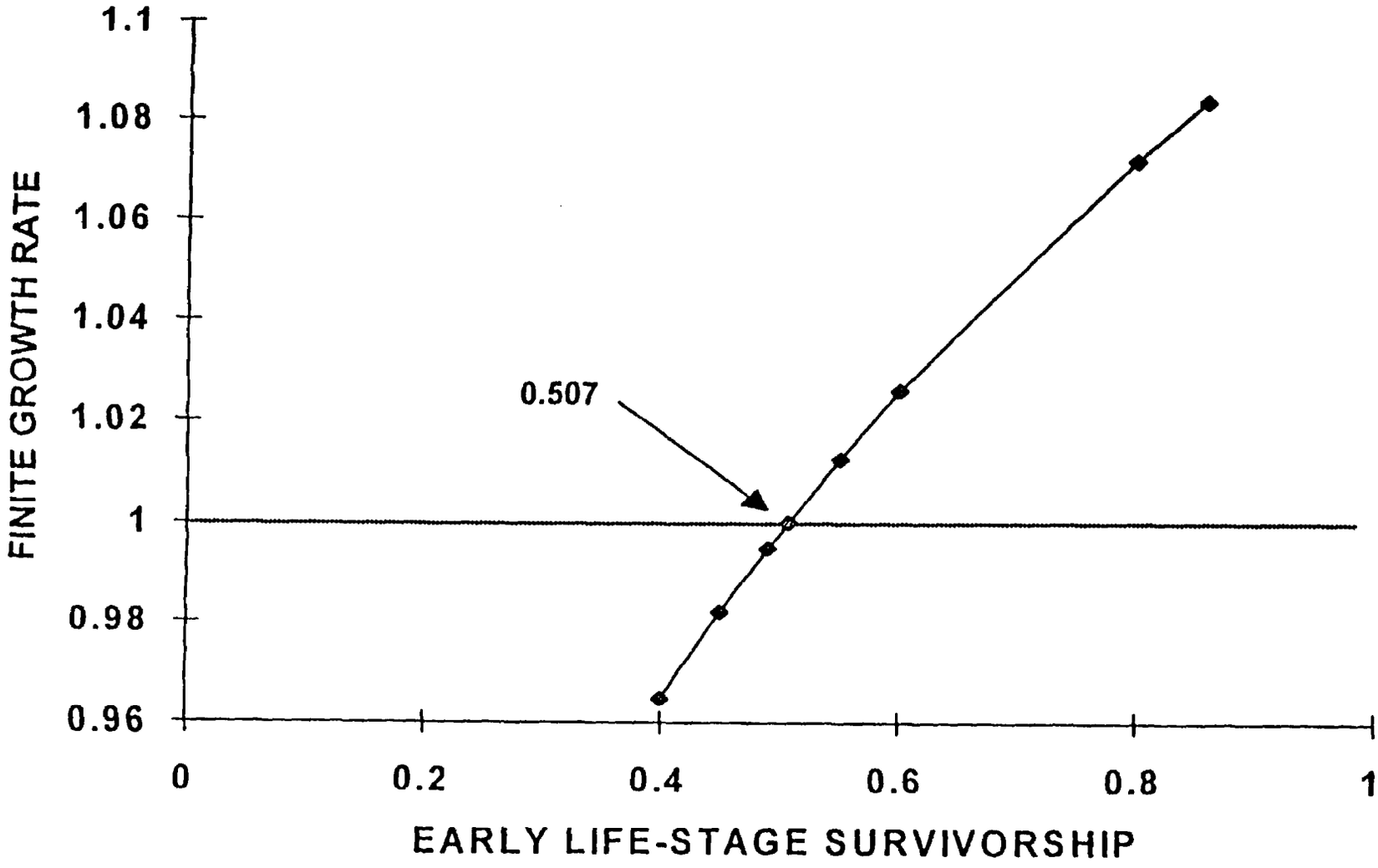
ESTABLISH BASIC POPULATION PARAMETERS

- o Age-specific survival and fertility
- o Model-based population estimation
- o Leslie Projection Matrix

TEST DESIGN

- o Test duration of 70 days
- o Regular non-destructive sampling
- o Examine mortality effects at 10-day intervals

Sensitivity to Early Life-Stage Survivorship



AMPHIPOD CHRONIC TESTS

WHY DO CHRONIC TESTS?

More sensitive than acute

More relevant ecological processes

Understand population biology

Determine sensitive life stages

EPA/COE guidance

Sediment Management Strategy

AMPHIPOD CHRONIC TESTS

RESEARCH GOAL

- Understand population biology
- Optimize design for:

  information content

  cost efficiency

AMPHIPOD CHRONIC TESTS

EARLY DESIGNS WITH *AMPELISCA*

- Population sampling approach:
  - initiate with ovigerous females
  - two generations
- Suspended sediments
- What we learned:
  - amphipods will reproduce in the laboratory
  - growth is a sensitive endpoint
  - developed crude population models
- Limitations:
  - high variability
  - no age standardization
  - not amenable to model-based approaches

MINIMUM REQUIREMENTS FOR TEST PROTOCOL

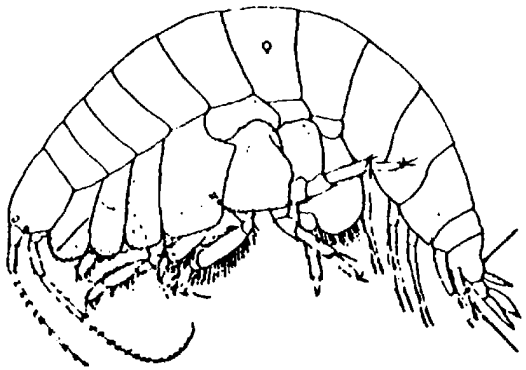
Responsive to chemical contaminants

Relatively sensitive

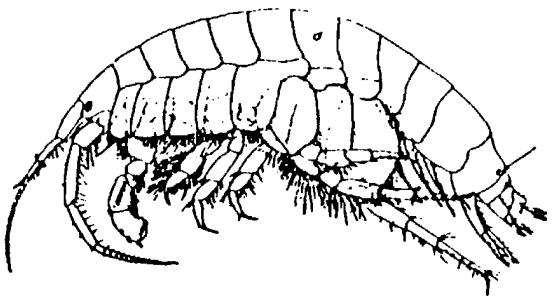
Intra- and interlaboratory variability low

Organism available

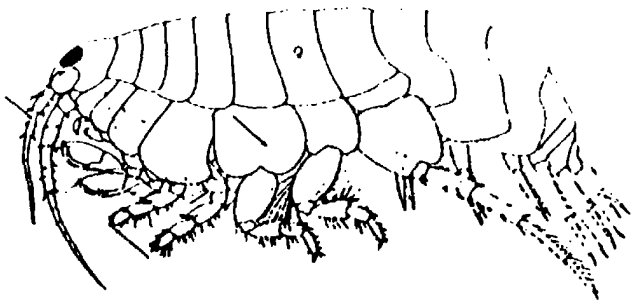
Well documented



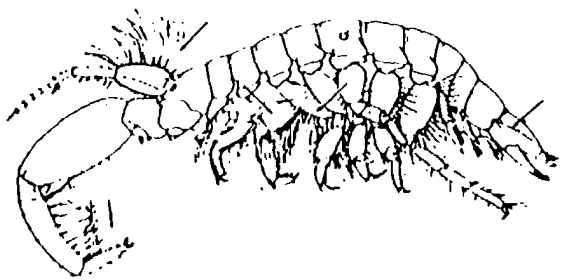
Ampelisca abdita



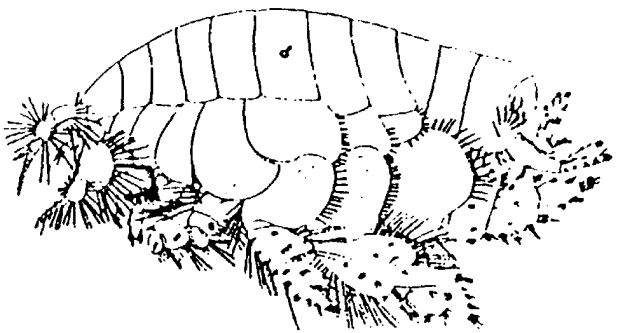
Leptocheirus plumulosus



Monoculodes edwardsi



Corophium lacustre

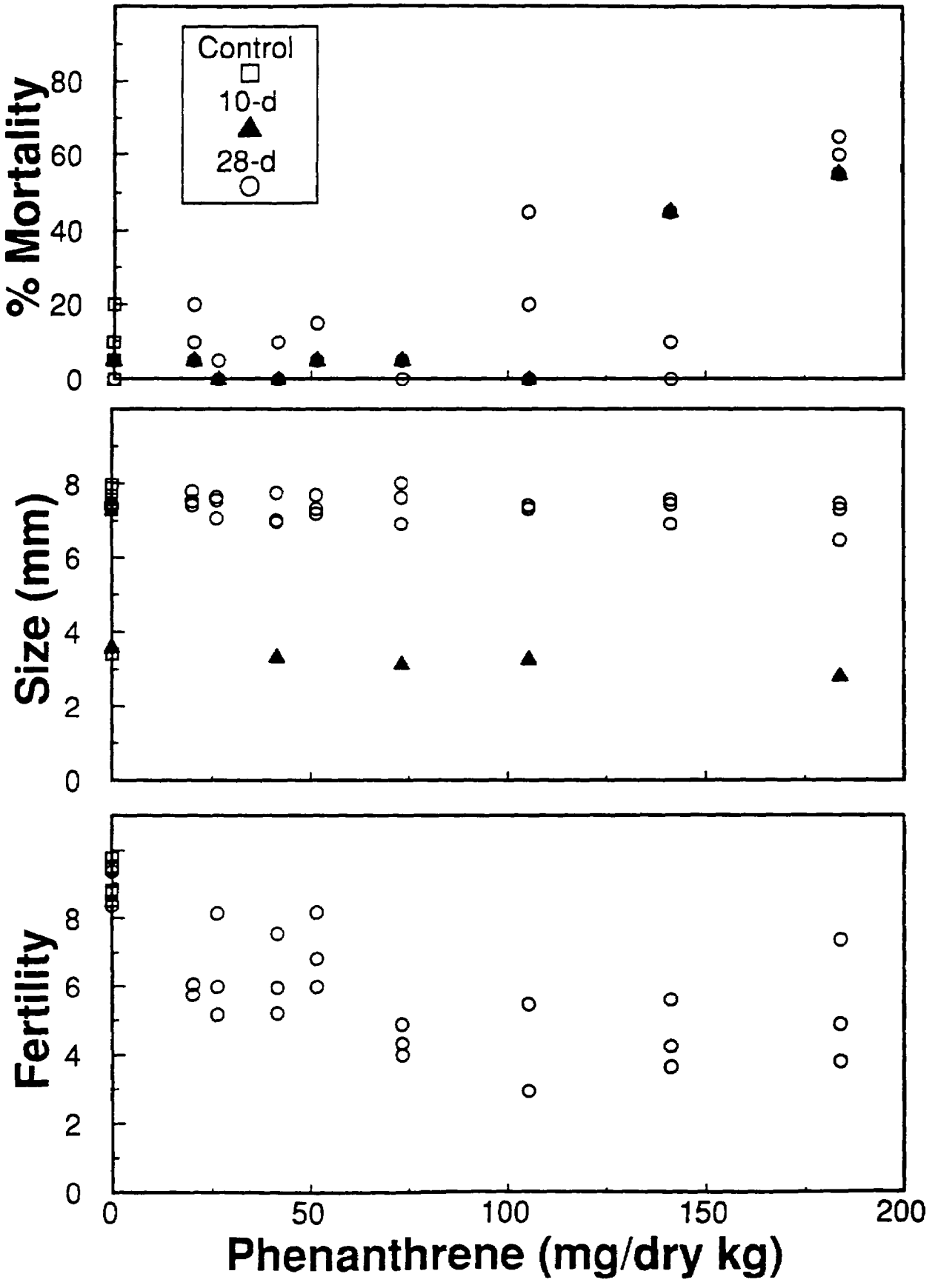


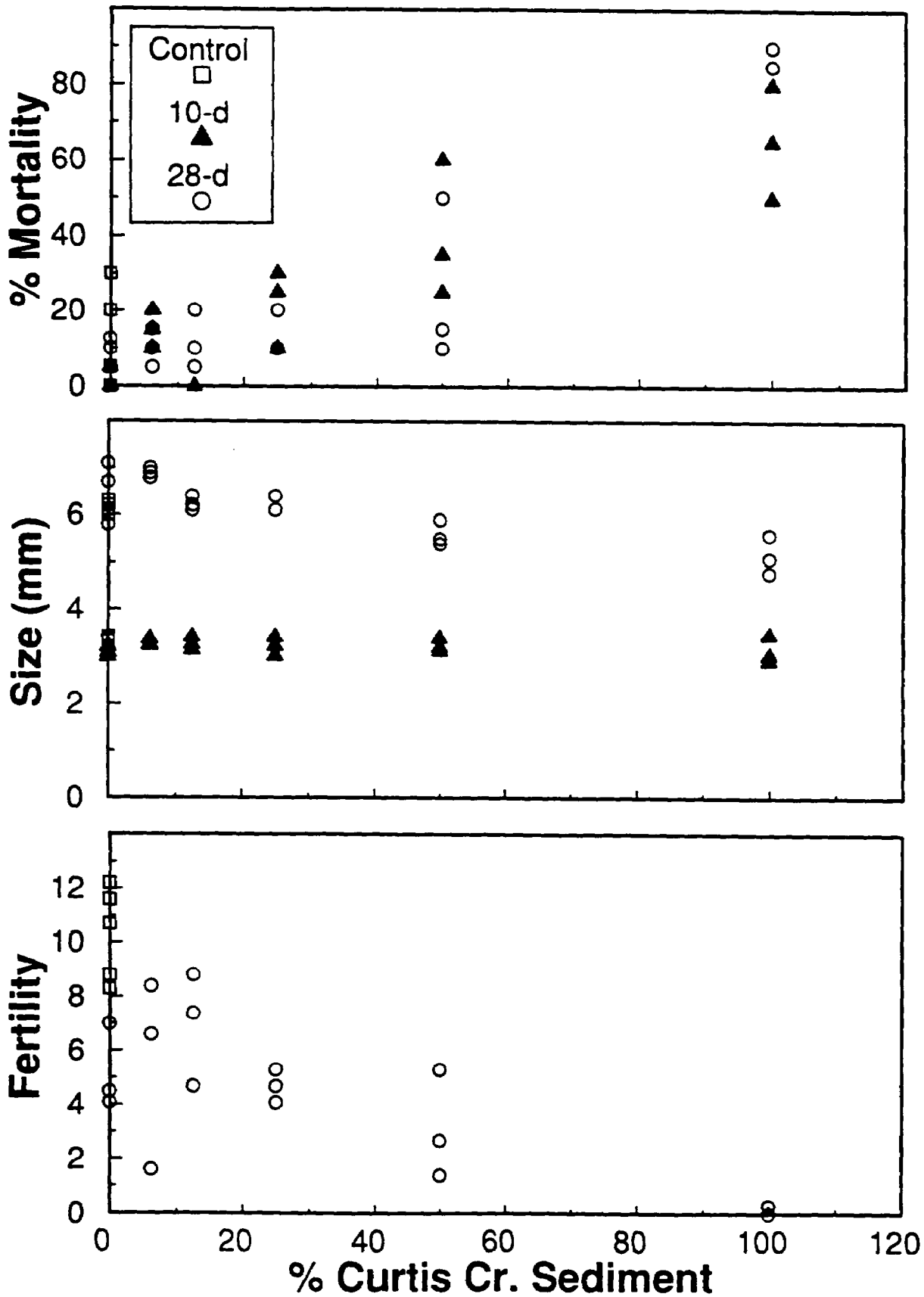
Lepidactylus dytiscus

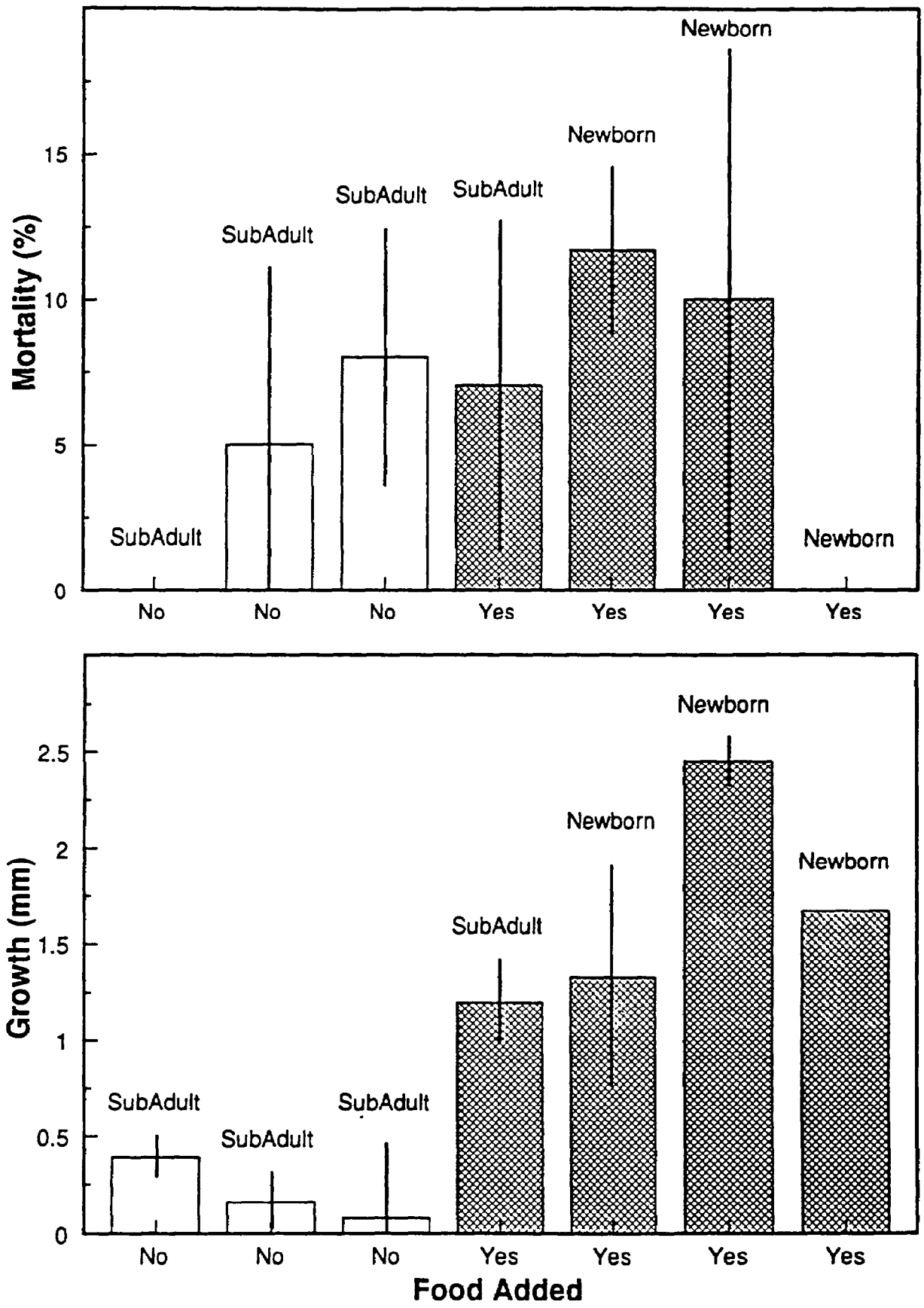
RELATIVE SENSITIVITY

96 h Exposures, Cd in Seawater

Species	Salinity (‰)	Temp (°C)	LC50 (Cd mg/L)	LC50 (Free Cd^{2+} ppm)
Ampelisca abdita	28	15	1.09	0.07
Lepidactylus dytiscus	28	15	6.86	0.47
	20	15	6.82	0.28
Leptocheirus plumulosus	28	20	2.79	0.19
(sub-adults)	28	15	9.80	0.67
	20	20	2.06	0.09
	20	15	13.37	0.56
Monoculodes edwardsi	20	15	<0.24	<0.02
Eohaustorius estuarius	28	15	11.41	0.78
	20	15	6.42	0.27
Rhepoxynius abronius	28	15	0.79	0.06


Control
10-d
28-d
% Mortality
0
20
40
60
80
Size (mm)
0
2
4
6
8
Fertility
0
2
4
6
8
0
50
100
150
200
Phenanthrene (mg/dry kg)


Control
10-d
28-d
% Mortality
0
20
40
60
80
Size (mm)
0
2
4
6
Fertility
0
2
4
6
8
10
12
0
20
40
60
80
100
120
% Curtis Cr. Sediment


Mortality (%)
0
5
10
15
SubAdult
SubAdult
SubAdult
SubAdult
Newborn
Newborn
Newborn
No
No
No
Yes
Yes
Yes
Yes
Growth (mm)
0
0.5
1
1.5
2
2.5
SubAdult
SubAdult
SubAdult
SubAdult
Newborn
Newborn
Newborn
No
No
No
Yes
Yes
Yes
Yes
Food Added

Rationale For Selecting *Leptocheirus plumulosus*

- **Endemic to Maryland's portion of Chesapeake Bay**
- **Success in identifying sediment toxicity with adult L. plumulosus in 10 d tests**
- **Exhibition of growth and reproduction under laboratory conditions**

SUMMARY OF TEST CONDITIONS FOR PARTIAL LIFE-CYCLE TEST

CONDUCTED WITH Leptocheirus plumulosus

† 1) ORIGIN OF TEST ANIMALS:	Obtained by isolating gravid ♀s in control sediment for ≤ 1 week. Offspring collected on 250 μm sieve are utilized in test. Size has ranged from 1.2 to 1.5 mm.
2) No. AMPHIPODS/CHAMBER:	20
3) VOLUME OF TEST CHAMBER:	1 L
4)†WATER:SEDIMENT RATIO:	4:1 (v:v)
5) PHOTOPERIOD:	16:8 (light:dark)
6)†WATER SOURCE:	Artificial seawater diluted with distilled and spring water
7)†TEST WATER TEMPERATURE:	20 ± 2°C
8)†TEST WATER SALINITY:	Ambient interstitial salinity of test sediment
9) TEST DURATION:	28 d; Static-renewal 2 X week
10)†TEST ENDPOINTS:	Survival, length, measures of reproduction
11) POSITIVE CONTROL:	Aqueous cadmium chloride @ 20°C and 20 ‰
12)†FEEDING REGIME:	6-12 mg TetraMin + Tetra Conditioning 3 X week
13)†CONTROL SEDIMENT:	Amphipod collection site sediment (Corsica River, Queen Anne's County, MD; 93% silt-clay)

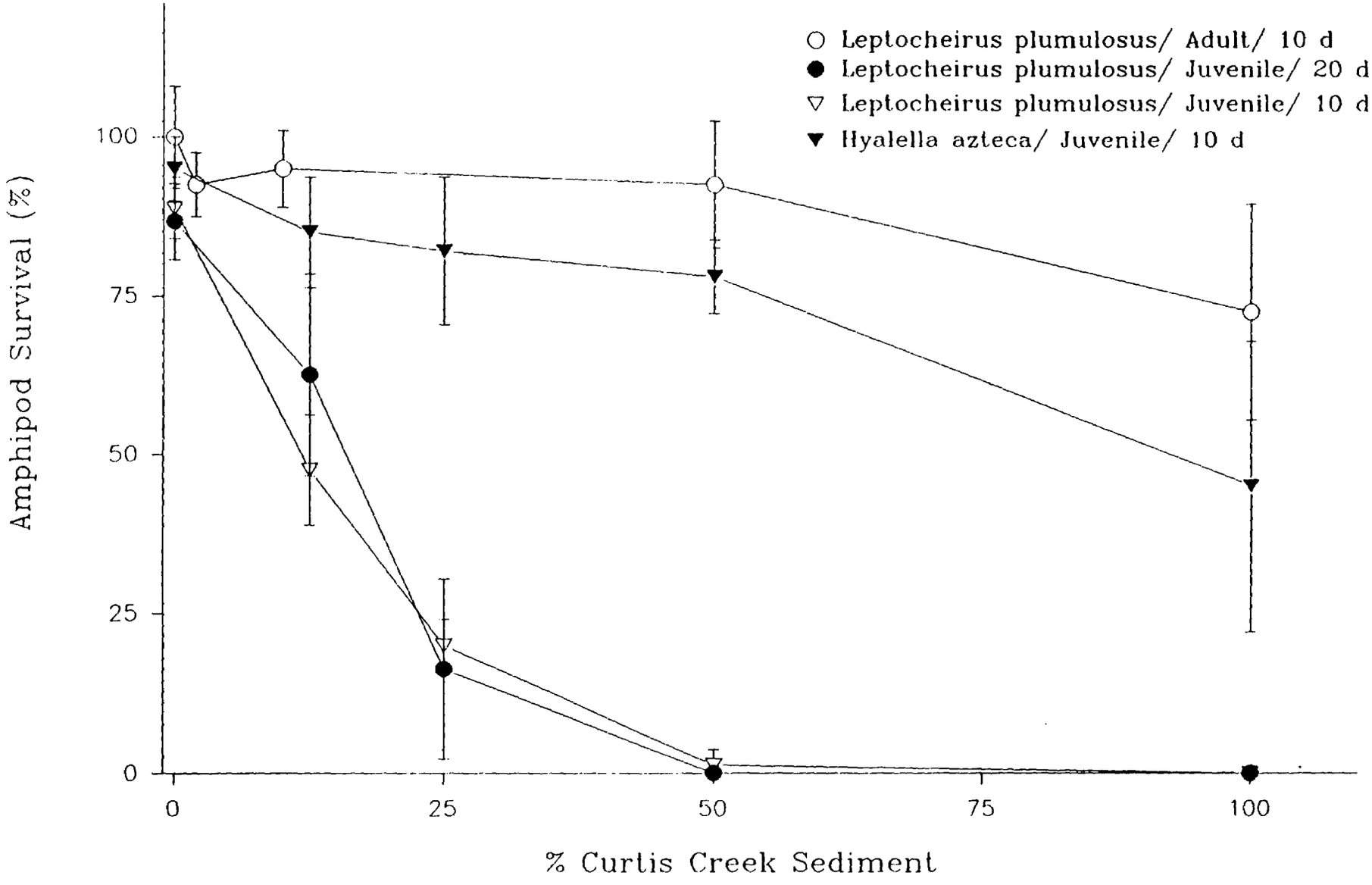
†: Different from EPA-Newport

Research Findings with Contaminated Sediments

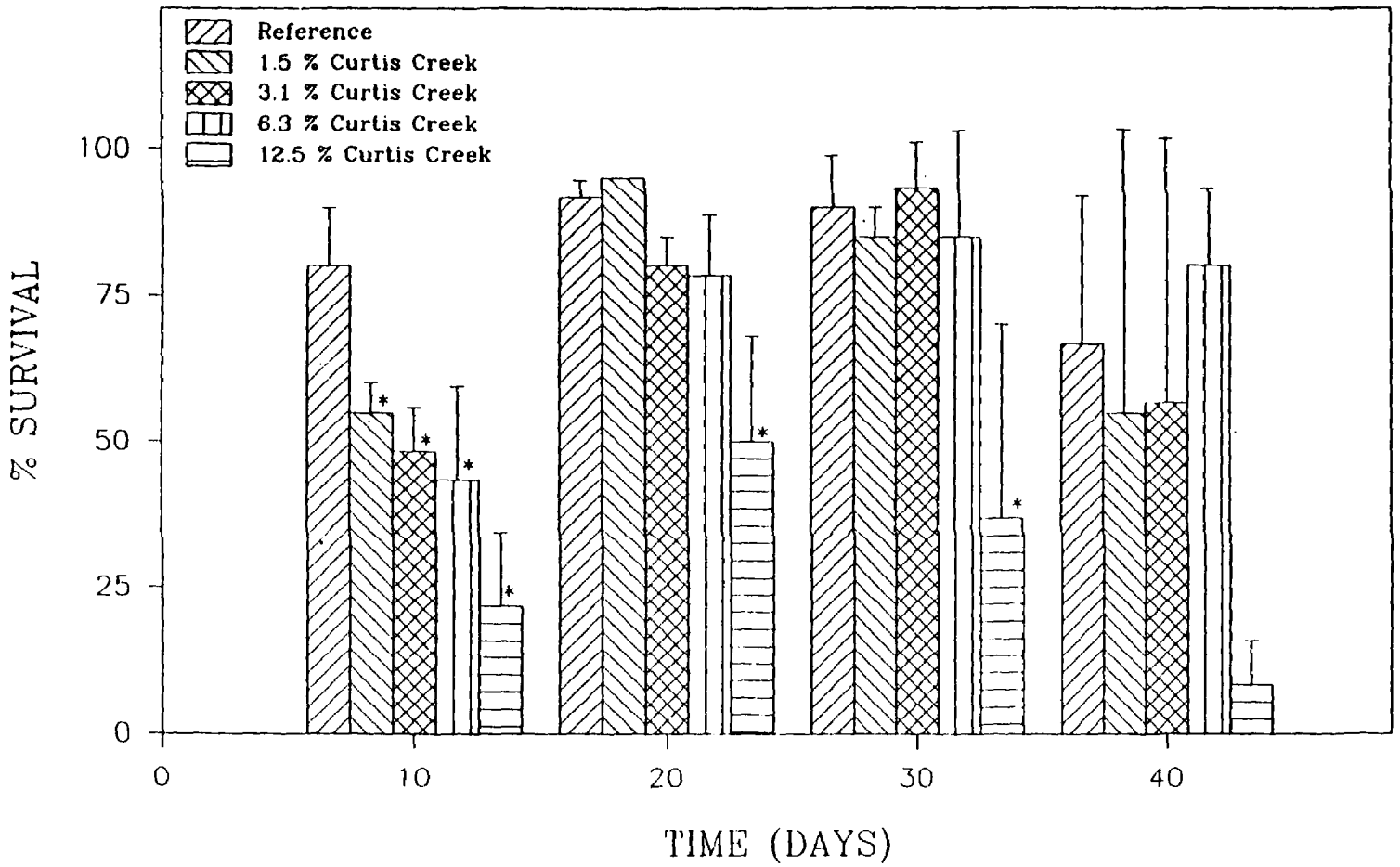
- **Comparative sensitivity to gradient of sediment contamin**
  Juvenile and adult *L. plumulosus* and juvenile *Hyalella az*

- **Evaluation of appropriate test endpoints and duration:**
  Juvenile *L. plumulosus* in sediment contamination gradien

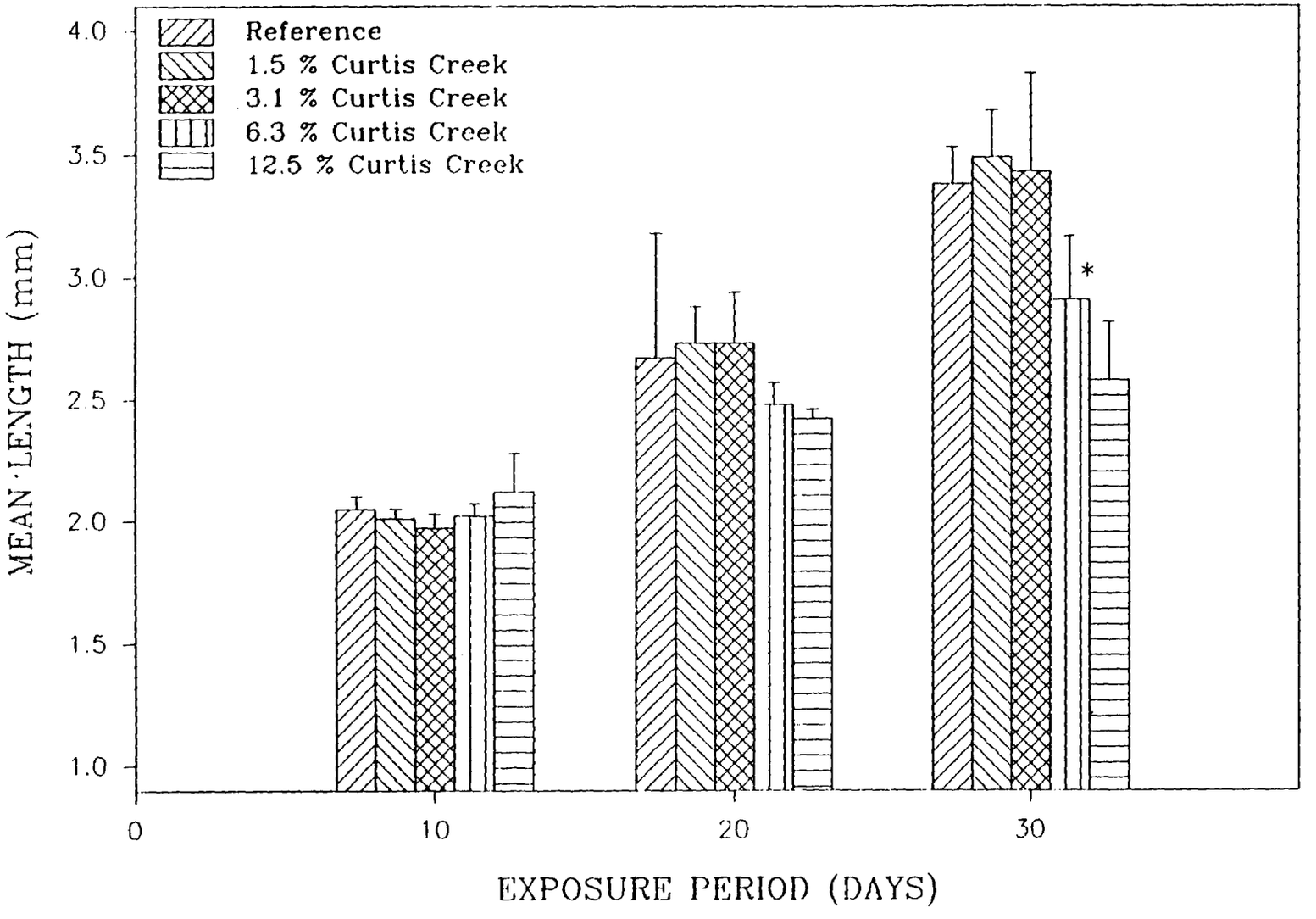
- **Evaluation of Non-toxicant variables:**
  Juvenile *L. plumulosus* in sediment contamination gradien
  under variable temperature and feeding regimes


Leptocheirus plumulosus/ Adult/ 10 d
Leptocheirus plumulosus/ Juvenile/ 20 d
Leptocheirus plumulosus/ Juvenile/ 10 d
Hyalella azteca/ Juvenile/ 10 d
Amphipod Survival (%)
100
75
50
25
0
0
25
50
75
100
% Curtis Creek Sediment

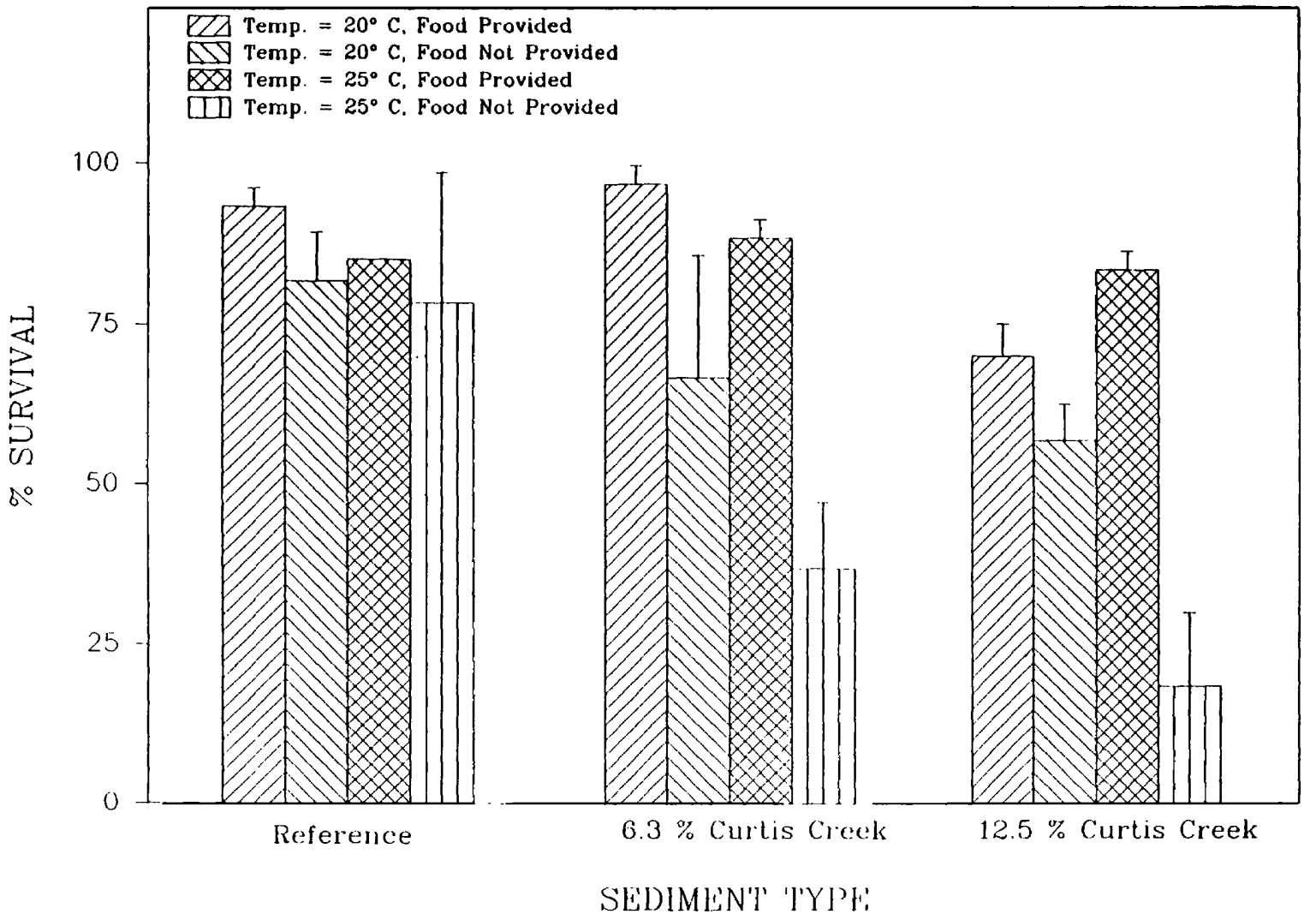
Leptocheirus plumulosus: Survival in Gradient of Sediment Contamination



Leptocheirus plumulosus: Growth in Gradient of Sediment Contamination



Leptocheirus plumulosus: Effect of Temperature and Feeding



APPENDIX A:

WORKSHOP AGENDA

Tiered Testing
Issues for Freshwater and Marine Sediments

Wednesday, September 16, 1992

8:30 **Introduction and Description of EPA Sediment Strategy**

Elizabeth Southerland, Workshop Moderator, U.S. EPA Office of Science and Technology

9:00 **Discussion of EPA Program Office Sediment Evaluation Needs**

Tom Armitage, U.S. EPA Office of Science and Technology

9:30 **Discussion of EPA Regional Sediment Evaluation Needs**

William Peltier, Environmental Services Division, U.S. EPA Region 4

10:00 **Break**

10:15 **Tiered Sediment Testing Conceptual Overview**

Elizabeth Southerland, U.S. EPA Office of Science and Technology

Tom Armitage, U.S. EPA Office of Science and Technology

10:45 **Summary of ASTM Activities on Freshwater and Marine Sediment Test Methods**

Chris Ingersoll, U.S. Fish and Wildlife Service National Fisheries Contaminant Research Center

11:15 **Discussion of Approaches for Test Standardization: Historical Perspective and Present Guidance**

Jim Lazorchak, U.S. EPA Environmental Monitoring and Systems Laboratory, Cincinnati

Bill Telliard, U.S. EPA Office of Science and Technology

12:15 **Lunch**

1:15 **Discussion of Desirable and Necessary Attributes for Freshwater Sediment Toxicity Tests, and the Use of Hyalella azteca and Chironomus tentans in Freshwater Sediments**

Allen Burton, Wright State University

John Giesy, Michigan State University, Department of Fisheries

Chris Ingersoll, U.S. Fish & Wildlife Service National Fisheries Contaminant Research Center

2:00 **Discussion of Desirable and Necessary Attributes for Marine and Estuarine Sediment Toxicity Tests, and the Use of Ampelisca abdita, Rhepoxynius abronius, Leptocheirus plumulosus, Eohaustorius estuarius in Marine and Estuarine Sediments**

Rick Swartz, U.S. EPA Environmental Research Laboratory, Pacific Division

3:15 **Break**

3:30 **Discussion of Desirable and Necessary Attributes for Freshwater, Marine, and Estuarine Bioaccumulation Test, and the Use of Neries and Macoma in Marine and Estuarine Sediments**

Peter Landrum, NOAA Great Lakes Environmental Research Laboratory

Henry Lee, U.S. EPA Environmental Research Laboratory, Pacific Division

4:45 **Discussion of the Use of Lumbriculus variegatus in Freshwater Sediments**

Gary Ankley, U.S. EPA Environmental Research Laboratory, Duluth

5:00 **Identification of Long Term Needs for Assessing Sediments**

Gary Ankley, U.S. EPA Environmental Research Laboratory, Duluth

Norm Rubinstein, U.S. EPA Environmental Research Laboratory, Narragansett

5:30 **Adjourn**

Break-Out Workgroup for Freshwater Sediment Issues

Thursday, September 17

8:00 am **Introduction and Overview of Day**

Moderator/Discussion Leader: Gary Ankley

- **General description of survey/summary of methods**
  Number of labs responding, who responded on which organisms, et cetera.
- **Discussion of issues for each method**

8:30 am **Development of a Standard Protocol for Testing Hyalella azteca**

Discussion Leader: Teresa Norberg-King
Rapporteur: Chris Ingersoll

- **Summary of culture methods used as reported in questionnaires (~10 min)**
- **Summary of test methods used as reported in questionnaires (~10 min)**
- **Proposed key issues for discussion**

10:15 am **Break**

10:30 am **Continuation of Discussion of Development of a Standard Protocol for Testing Hyalella azteca**

11:30 am **Development of a Standard Protocol for Chironomus tentans**

Discussion Leader: Bob Hoke
Rapporteur: Jody Kubitz

- **Summary of culture methods used as reported in questionnaires (~10 min)**
- **Summary of test methods used as reported in questionnaires (~10 min)**
- **Proposed key issues for discussion**

12:15 pm **Lunch**

1:30 pm **Continuation of Chironomus tentans**

2:30 pm **Development of a Standard Protocol for Lumbriculus variegatus**

Discussion Leader: Peter Landrum
Rapporteur: Allen Burton

- **Summary of culture methods used as reported in questionnaires (~10 min)**
- **Summary of test methods used as reported in questionnaires (~10 min)**
- **Proposed key issues for discussion**

3:30 pm **Break**

3:45 pm **Continuation of Lumbriculus variegatus**

5:00 pm **General Adjournment**

5:30 pm **Meeting of Discussion Leaders, Moderators, and Rapporteurs to Write Conclusions**

Break-Out Workgroup for Marine and Estuarine Sediment Issues

Thursday, September 17

8:00 am **Overview of Day**

- **Discussion of Issues for Each Method**

Norm Rubinstein, U.S. EPA Environmental Research Laboratory, Narragansett

8:30 am **Development of a Standard Acute Amphipod Protocol**

- **Summary of methods used**
- **Applications of the test**
- **Minimum requirements for the method**
- **Technical issues**

Rick Swartz, U.S. EPA Environmental Research Laboratory, Pacific Division

10:15 am **Break**

10:30 am **Development of a Standard Chronic Amphipod Protocol**

- **Summary of methods used**
- **Applications of the test**
- **Minimum requirements for the method**

John Scott, SAIC

Ted DeWitt, AScI

12:15 pm **Lunch**

1:30 pm **Continuation of Chronic Protocol Discussion**

2:30 pm **Development of a Standard Bioaccumulation Protocol**

- **Summary of methods used**
- **Applications of the test**
- **Minimum requirements for the method**
- **Technical issues**

Henry Lee, U.S. Environmental Research Laboratory, Pacific Division

3:30 pm **Break**

3:45 pm **Continuation of Bioaccumulation Protocol Discussion**

5:00 pm **Development of Other Test Methods and Standard Protocols**

5:30 pm **Adjourn**

Tiered Testing
Issues for Freshwater and Marine Sediments

Friday, September 18

9:00 am **Overview of Day**

Elizabeth Southerland, Workshop Moderator, U.S. EPA Office of Science and Technology

9:15 am **Report of Issues Covered in Marine Methods Session and Discussion of Conclusions/Next Steps**

Rick Swartz, U.S. EPA Environmental Research Laboratory, Pacific Division

10:15 am **Report of Issues Covered in Freshwater Methods Session and Discussion of Conclusions/Next Steps**

Gary Ankley, U.S. EPA Environmental Research Laboratory, Duluth

11:15 am **Workshop Summary and Wrap-up**

Elizabeth Southerland, Workshop Moderator, U.S. EPA Office of Science and Technology

11:45 am **Adjourn**

APPENDIX B:

SEDIMENT TOXICITY TESTS UNDER DEVELOPMENT BY ENVIRONMENT CANADA

DEVELOPMENT OF A 10-DAY MARINE/ESTUARINE AMPHIPOD ASSAY FOR SEDIMENT TOXICITY IN SUPPORT OF THE CANADIAN OCEAN DUMPING PROGRAM (CEPA, PART VI).

D.J. McLeay, S.C. Yee, K.G. Doe and L.M. Porebski, McLeay Associates Limited, West Vancouver, British Columbia, Environment Canada, and Aquatic Toxicity Laboratory, North Vancouver, British Columbia, and Environment Canada, Laboratory Division, Dartmouth, Nova Scotia, and Environment Canada, Office of Waste Management, Environment Canada, Ottawa, Ontario.

ABSTRACT

Beginning in 1988, Environment Canada commenced the development of a test method for measuring the acute toxicity of sediment samples, using a number of marine or estuarine sediment-burrowing amphipods common to Canada's coastal waters. The evolution of this toxicity test included five series of inter-laboratory assessments using various candidate test organisms, sediment samples, and a reference toxicant. These studies are reviewed briefly. Additionally, the test method and its applications are summarized.

INTRODUCTION

The Ocean Dumping Control Act has been consolidated into Part VI of the Canadian Environmental Protection Act (CEPA). The Act requires valid ocean dumping permits before dumping of any substance at sea is allowed. Biological testing and assessment can be an integral component of this permit process (Sergy, 1988; Anthony, 1991; Porebski, 1991). In order for Environment Canada to perform its regulatory responsibilities associated with this Act, biological screening tests using marine or estuarine organisms may be required to determine if material is suitable for unconfined open-water disposal, and to perform environmental-effects monitoring at dump sites. Interim contaminant testing guidelines for ocean disposal, associated with Environment Canada's Ocean Dumping Control Program, specify several toxicity tests for use in screening materials. The 10-day assay for sediment toxicity, using one or more species of estuarine or marine amphipods common to Canada's coastal waters, is included on the lists of biological screening tests (Environment Canada, 1990a, 1990b).

In consideration of the above, Environment Canada's Atlantic and Pacific & Yukon regional laboratories commenced inter-laboratory studies in late 1988, using a number of marine or estuarine sediment-burrowing amphipods and various samples of control, reference or test (contaminated) sediments. The objectives of this testing program were twofold: (1) to study several candidate species of amphipods, selecting those suitable for use in acute lethality tests with samples of sediment or other test material; and (2) to evaluate conditions, procedures and biological endpoints appropriate for use in a standard biological test method to be developed to meet Environment Canada's testing requirements in this respect. The past status of this and other biological test methods under development on behalf of Environment Canada has been reported (McLeay et al., 1991a).

Following is a brief summary of the inter-laboratory studies with marine or estuarine amphipods, performed to date by Environment Canada's Pacific & Yukon and Atlantic regional laboratories.[1] Also presented is a list of those species of infaunal amphipods presently recommended for use in Environment Canada's draft biological test method "Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods" (Environment Canada, 1991). Tentative checklists of recommended conditions and procedures for holding and acclimating amphipods, and for testing them in 10-day static assays, are provided. Finally, some of the applications of this biological test method are indicated.

LABORATORY EVALUATION AND DEVELOPMENT OF TEST METHOD

In 1988, Environment Canada's Atlantic and Pacific & Yukon regional laboratories undertook a preliminary (Phase-I) evaluation of the 10-day sediment assay, using only Rhepoxynius abronius (McLeay et al., 1989). The effects of holding amphipods in control sediment for periods of up to 81 days, on their 10-day survival and subsequent reburial rates under test conditions (control sediment only), were investigated. Their acute tolerance (96-h LC50/EC50) to the reference toxicant cadmium chloride (seawater-only exposures) was monitored during the prolonged holding period. Although 109-day survival and reburial rates were acceptable ($\geq$90%) in all trials, the reference toxicant tests indicated a declined tolerance of these organisms with extended $\geq$13 days) periods of holding in the laboratory.

The second (Phase-II) inter-laboratory study (McLeay et al., 1991b) measured and compared the 10-day survival, emergence and reburial rates for a population of Rhepoxynius abronius exposed to control,[2] reference,[3] or test[4] sediment. At each laboratory, survival and reburial rates in control sediment were high ($\geq$90%). No consistent differences in these endpoints were caused by exposure to reference or test sediments, indicating that neither were highly toxic. However, increased rates of emergence from the test sediment were observed.

---

[1]Basic test conditions and procedures used for the 10-day static assays were according to Swartz et al. (1985) and ASTM (1990).

[2]Control sediment is clean sediment taken from the site where the test organisms were collected, and intended for use in the 10-day test with amphipods. This sediment must contain no test material. It is used to determine the absence of measurable toxicity due to basic test conditions (e.g., temperature, health or handling of test organisms).

[3]Reference sediment is a field-collected sample of sediment, taken from a site thought to be relatively free of contaminants (i.e., "clean" sediment), and intended for use in the 10-day test with amphipods. It is often collected from a site within the general vicinity of a test sediment, and is frequently selected for biological testing because of its geochemical similarity (e.g., particle size, compactness, total organic content) to the test sediment(s).

[4]Test sediment is a field-collected sample of solid-phase sediment, taken from a site thought to be contaminated with one or more chemicals, and intended for use in the 10-day test with amphipods. In this study, the sample of test sediment was collected from the vicinity of a B.C. coastal pulp mill discharging bleached kraft mill effluent.

A Phase-III study was performed by each laboratory using both Rhepoxynius abronius and Corophium volutator as test organisms (McLeay et al., 1991b). For each species, ten-day survival and subsequent reburial rates were determined for three clean[5] (control or reference) sediments and three test sediments. The clean sediments varied appreciably in grain size, with silt/clay contents of 1%, 82% or 99%. Tests with R. abronius showed highest survival rates (96% and 100%) for control sediment, and lowest survival rates (43% and 78% for the reference sediment containing 99% fines (silt and clay). R. abronius survival rates for the three test sediments ranged from 72 to 93%. Unlike these findings, survival rates for Corophium volutator were high (93% and 97%) in the extremely fine-grained reference sediment. For both species, reburial and/or survival data[6] showed no consistent response for any of the test sediments examined, although emergence rates indicated an avoidance response to one of the test sediments.

A fourth inter-laboratory assessment,[7] using six species of marine or estuarine infaunal amphipods[8] common to Canada's coastal waters, was undertaken during late 1990 and early 1991 (Paine and McPherson, 1991a). The objectives of this (Phase-IV) study were to determine and compare the relative sensitivity of each of the six test species to four test sediments, two reference sediments (fine-grained and coarse-grained), and control sediments (one for each species). For the four test sediments examined, each of the six species of amphipods used in these assays distinguished the same two sediments as clearly toxic, and the remaining two as marginally or not toxic. Percentage survival at 10 days was the most useful biological endpoint; little if any additional information was obtained using the other two endpoints (% emergence, % of survivors that did not rebury in control sediment within 1 h following test completion). E. washingtonianus and R. abronius were most sensitive to the test sediments; C. volutator and E. estuarius were least sensitive. Two of the six species studied (E. washingtonianus and A. virginiana) showed unacceptably low (<90%) 10-day survival rates in control sediment. Depending on species, grain-size effects were minimal or not evident. This study also demonstrated that, if care is taken, amphipods can be shipped across the country without influencing the test results.

Additional studies were performed by Environment Canada's Atlantic regional laboratory in early 1991 to assess the laboratory hardiness and worth of Amphiporeia virginiana as a test organism (Doe, 1991). Two populations of field-collected specimens were tested for 10-day survival rates in control sediment only. Animals were acclimated and tested at temperatures of 10 or 15 C (Series 1); and at 5, 10 or 15 C (Series 2). Results from these studies showed that acceptable (≥90%) 10-day survival rates could be obtained at 5 or 10 C, but not at 15 C. Seasonally-cold (2 to 4 C) seawater temperatures at collection sites likely contributed to these findings.

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[5]Clean sediment is sediment (e.g., control or reference sediment) that does not contain concentrations of contaminants which cause discernible distress to the test organisms or reduce their survival in 10-day assays.

[6]Since C. volutator do not rebury readily in control sediment within 1 hour, this biological endpoint cannot be determined for this species.

[7]Participating laboratories included Environment Canada's Atlantic and Pacific & Yukon regional laboratories, and EVS Consultants Ltd.

[8]Test organisms were Rhepoxynius abronius, Foxiphalus xiximeus, Eohaustorius estuarius, Eohaustorius washingtonianus, Corophium volutator, and Amphiporeia virginiana.

A fifth inter-laboratory[9] appraisal of the 10-day test for sediment toxicity, using multiple species of marine or estuarine amphipods, was conducted during June 1991 (Paine and McPherson 1991b). For this study, seven species of infaunal amphipods[10] were collected and examined for their laboratory adaptability and sensitivity to each of the three test sediments. Biological endpoints (% survival and % emergence at 10 days; % reburial of survivors in control sediment at test end) were determined for each species in these sediments as well as in a fine-grained (79% silt-clay) reference sediment and respective control sediments. Assays with Amphiporeia virginiana were performed at both 10 and 15 C; all other species were tested at 15 C only. Unlike the previous (Phase-IV) inter-laboratory study, none of the three test sediments used in this study were highly toxic to any of the species of amphipods examined. Once again, ten-day survival rates in control sediment were unacceptably low (,90%) for Amphiporeia virginiana (both temperatures) and Eohaustorius washingtonianus, but acceptable for all other species studied including Leptocheirus pinguis. As in the Phase-IV study, % survival at 10 days was the most useful biological endpoint, with little if any additional information provided by the secondary endpoints (% emergence, % of survivors not reburying in control sediment at test end).

RECOMMENDED TEST SPECIES

Recent attempts have been made to identify suitable Canadian collection sites,[11] and to collect the following species of amphipods from Canadian coastal waters, in order to evaluate their worth as candidate test organisms in 10-day sediment assays:

Pacific Coast	Atlantic Coast
Monoculodes spinipes	Rhepoxynius hudsoni
Grandifoxus grandis	Phoxocephalus holbolli
	Ampelisca abdita
	Ampelisca vadorum
	Amphiporeia lawrenciana
	Pontoporeia femorata

The absence or relative scarcity of these species at the Canadian collection sites investigated, prevented their inclusion in the present test program.

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[9]Environment Canada's Atlantic and pacific & Yukon regional laboratories.

[10]Test organisms included Rhepoxynius abronius, Foxiphalus xiximeus, Eohaustorius estuarius, Eohaustorius washingtonianus, Corophium volutator, Amphiporeia virginiana, and Leptocheirus pinguis.

[11]Specimen collections held by the National Museum of Natural Sciences (Ottawa, Ontario) were examined by Dr. E.L. Bousfield, together with historical records available to him (e.g., Bousfield, 1990a, b; Bousfield, 1991; Bousfield and Laubitz, 1972). Based on these and other distributional and life-history information, initial recommendations were made for prospective test specimens.

Based on the results of the laboratory studies mentioned previously (see section "Laboratory Evaluation and Development of Test Method"), as well as those for other 10-day assays performed with the candidate species of infaunal amphipods under consideration by Environment Canada, the following species of marine or estuarine amphipods are presently recommended for use in 10-day static sediment assays (Environment Canada, 1991):

Recommended Pacific Species	Recommended Atlantic Species
Rhepoxynius abronius	Corophium volutator
Foxiphalus xiximeus	Leptocheirus pinguis
Eohaustorius estuarius	

Additional studies with Amphiporeia virginiana and Eohaustorius washingtonianus are required to demonstrate that satisfactory survival rates in control sediment can be achieved for these species, before they can be recommended by Environment Canada as standard test organisms.

RECOMMENDED CONDITIONS AND PROCEDURES FOR ACCLIMATING AND TESTING AMPHIPODS

Environment Canada's draft biological test method (Environment Canada, 1991) provides details regarding conditions and procedures for holding and acclimating amphipods to be used in 10-day assays, as well as those necessary to perform the test in a standardized manner. The test methods of Swartz et al. (1985) and ASTM (1990) formed the basis for Environment Canada's (1991) acute test for sediment toxicity using marine or estuarine amphipods common to Canadian coastal waters. Acclimation and test procedures recommended in Environment Canada (1991) are reproduced here (see Tables 1 and 2). Since this biological test method is not yet finalized and approved by Environment Canada, these procedures and conditions are subject to change.

Table 1 Checklist of Recommended Conditions and Procedures for Holding and Acclimating Amphipods[12]

Source of amphipods:	-	Collected subtidally or intertidally from clean sediment
Life stage:	-	juveniles or young adults, 3-10 mm length (depending on species)
Sorting:	-	Sieve through 1.0-mm screen to confirm species and select appropriate size; use seawater within 2 C and 2 ppt salinity of the seawater in transport container
Holding sediment:	-	Control sediment, 2-4 cm in depth, previously sieved through 0.5-mm mesh
Holding seawater:	-	Reconstituted or clean natural seawater
Acclimation conditions:	-	Salinity of seawater same as that for overlying seawater in test; temperature normally 15 $\pm$ 2 C; dissolved oxygen $\geq$ 90% of air saturation; temperature, salinity, and dissolved oxygen measured and recorded daily
Lighting:	-	Constant overhead illumination, $\geq$ 100 lux at surface of sediment in holding/acclimation container(s)
Feeding:	-	None
Duration of acclimation:	-	3 to 10 days
Health criteria:	-	Select amphipods able to bury quickly in control sediment; remove inactive amphipods that have emerged from sediment or do not bury; discard population if $\geq$5 % dead or emerged and inactive during 48-h period preceding test

[12]From Environment Canada's (1991) draft biological test method - subject to change.

APPENDIX C:

FRESHWATER SURVEYS

Culturing Questionnaire

Species: _____
Laboratory: _____
Contact: _____

1. Is the culture intermittent or continuous? If continuous, how long have animals been in culture at your facility?

2. What was original source and approximate date you started the culture?

3. Have the animals been taxonomically identified? If so, when and by whom?

4. What records on culture animals are maintained?

	circle one		Frequency
parental survival	yes	no	_____
age of brood animals	yes	no	_____
temperature	yes	no	_____
dissolved oxygen	yes	no	_____
pH	yes	no	_____
quality/age of foods	yes	no	_____
frequency of new culture chambers	yes	no	_____

5. What is source of the water used for culturing? List all used.

6. What are the characteristics of each water?

water	hardness	alkalinity	pH	conductivity

7. Have you used any reconstituted waters for culturing? If so, are they successful? If no, what problems did you experience?

8. What foods have you tried to culture the animals? What is your choice of food for routine culture?

9. How long has this regime been used?

10. Do you culture under controlled temperature ($\pm 2°C$) and lighting? If so, specify conditions.

11. What substrates have you used for culturing? Please list types/quantities evaluated and current choice.

12. Do you conduct any reference toxicant tests with this organism?

    A. If so, with what toxicant? What duration and was test conducted with or without sediment? If with sediment, specify source/type.

    B. Have results been reproducible? Have any control charts been established?

13. Do you feel reference toxicant tests are relevant for monitoring the adequacy/performance of the culture organisms?

14. Provide any additional information or comments that are pertinent to this species below:

Testing Questionnaire

Species: _____
Laboratory: _____
Contact: _____

1. Describe test (toxicity, bioaccumulation).

2. Are the test organisms cultured at your laboratory? If yes, provide description in Attachment 1. If no, please provide details of how test animals are obtained.

3. What is the "routine" test performed with this organisms at your laboratory? Select the appropriate time frame.

   _____ 4-d _____ 7-d _____ 10-d _____ 14-d _____ 21-d
   _____ other, specify _____

4. Do you use known age or size or unknown age or size of organisms to initiate tests? What is known age or size? Specify age or size. How do you obtain these known age/size organisms?

5. Is it important that animals are a minimum age (or size) for sediment:water exposures to ensure recovery (of organisms or sediments)?

6. Have you specified a certain test design of sediment:water? If so, what and why?

7. What is the renewal frequency of the overlying water for the duration of the test? What procedure is used to renew the water?

8. Do you feed during the test? Is it the same rate and frequency as in the culture? If not specify what and why.

9. What statistical analyses are performed on the data? Cite specific procedures and types of statistical analysis.

10. What are the test endpoints? How do you express the effect?

11. Have you conducted any reference toxicant tests in your laboratory sediment:water exposures? If yes, please identify chemicals and explain general trend of test results. Manuscripts can be attached.

12. What water quality characteristics of the overlying water are measured during the test? How often are these parameters measured?

13. Provide any additional information or comments that are pertinent to this species below:

PLEASE PROVIDE DATA IN SPACE BELOW
CULTURE CONDITIONS FOR HYALELLA AZTECA

CULTURE CONDITION	CONDITION USED BY LABORATORY
1. Culture type (static or renewal) (specify rate)	
2. Temperature:	
3. Light quality:	
4. Light intensity:	
5. Photoperiod:	
6. Culture chamber size:	
7. Culture water volume:	
8. Frequency of starting new culture	
9. Renewal of culture water:	
10. Removal of offspring (frequency):	
11. Age of restart organisms:	
12. No. organisms/culture chamber:	
13. No. of culture tanks:	
14. Feeding regime:	
15. Substrate used:	
16. Chamber cleaning:	
17. Aeration:	
18. Culture water:	

PLEASE PROVIDE DATA IN SPACE BELOW
CULTURE CONDITIONS FOR CHIRONOMUS TENTANS

CULTURE CONDITION	CONDITION USED BY LABORATORY
1. Culture type (static or renewal) (specify rate)	
2. Temperature:	
3. Light quality:	
4. Light intensity:	
5. Photoperiod:	
6. Culture chamber size:	
7. Culture water volume:	
8. Frequency of starting new culture	
9. Renewal of culture water:	
10. Removal of offspring (frequency):	
11. Age of restart organisms:	
12. No. organisms/culture chamber:	
13. No. of culture tanks:	
14. Feeding regime:	
15. Substrate used:	
16. Chamber cleaning:	
17. Aeration:	
18. Culture water:	

PLEASE PROVIDE DATA IN SPACE BELOW
CULTURE CONDITIONS FOR LUMBRICULUS VARIEGATUS

CULTURE CONDITION	CONDITION USED BY LABORATORY
1. Culture type (static or renewal) (specify rate)	
2. Temperature:	
3. Light quality:	
4. Light intensity:	
5. Photoperiod:	
6. Culture chamber size:	
7. Culture water volume:	
8. Frequency of starting new culture	
9. Renewal of culture water:	
10. Removal of offspring (frequency):	
11. Age of restart organisms:	
12. No. organisms/culture chamber:	
13. No. of culture tanks:	
14. Feeding regime:	
15. Substrate used:	
16. Chamber cleaning:	
17. Aeration:	
18. Culture water:	

PLEASE PROVIDE INFORMATION IN SPACES BELOW IF APPROPRIATE
CONDITIONS FOR HYALELLA AZTECA SEDIMENT TOXICITY TESTS

TEST CONDITION	CONDITION USED BY LABORATORY
1. Test type (static or renewal) (specify rate)	
2. Test duration:	
3. Temperature:	
4. Light quality:	
5. Light intensity:	
6. Photoperiod:	
7. Test chamber size:	
8. Test sediment volume:	
9. Overlying water volume: chamber:	
10. Age of test organisms:	
11. Size of test organisms:	
12. No. organisms/test chamber:	
13. No. replicate test chambers/sediment:	
14. No. organisms/sediment:	
15. Feeding regime:	
16. Test chamber cleaning:	
17. Aeration:	
18. Overlying water quality characteristics:	
19. Test acceptability criterion:	
(1) minimum control survival	
(2) length or weight minimum criteria:	
20. Endpoint(s)	

NOTE: If an item listed does not seem pertinent, please explain why.

PLEASE PROVIDE INFORMATION IN SPACES BELOW IF APPROPRIATE
CONDITIONS FOR CHIRONOMUS TENTANS SEDIMENT TOXICITY TESTS

TEST CONDITION	CONDITION USED BY LABORATORY
1. Test type (static or renewal) (specify rate)	
2. Test duration:	
3. Temperature:	
4. Light quality:	
5. Light intensity:	
6. Photoperiod:	
7. Test chamber size:	
8. Test sediment volume:	
9. Overlying water volume: chamber:	
10. Age of test organisms:	
11. Size of test organisms:	
12. No. organisms/test chamber:	
13. No. replicate test chambers/sediment:	
14. No. organisms/sediment:	
15. Feeding regime:	
16. Test chamber cleaning:	
17. Aeration:	
18. Overlying water quality characteristics:	
19. Test acceptability criterion:	
(1) minimum control survival	
(2) length or weight minimum criteria:	
20. Endpoint(s)	

NOTE: If an item listed does not seem pertinent, please explain why.

PLEASE PROVIDE INFORMATION IN SPACES BELOW IF APPROPRIATE
CONDITIONS FOR LUMBRICULUS VARIEGATUS SEDIMENT TOXICITY TESTS

TEST CONDITION	CONDITION USED BY LABORATORY
1. Test type (static or renewal) (specify rate)	
2. Test duration:	
3. Temperature:	
4. Light quality:	
5. Light intensity:	
6. Photoperiod:	
7. Test chamber size:	
8. Test sediment volume:	
9. Overlying water volume: chamber:	
10. Age of test organisms:	
11. Size of test organisms:	
12. No. organisms/test chamber:	
13. No. replicate test chambers/sediment:	
14. No. organisms/sediment:	
15. Feeding regime:	
16. Test chamber cleaning:	
17. Aeration:	
18. Overlying water quality characteristics:	
19. Test acceptability criterion:	
(1) minimum control survival	
(2) length or weight minimum criteria:	
20. Endpoint(s)	

NOTE: If an item listed does not seem pertinent, please explain why.

General Culturing Issues

Substrate
Density
Water
Flow-through vs. Static
Feeds/Feeding
Genetic drift/stream differences
Known age systems
Nuisance organisms
Light/photoperiod
Temperature
QA/QC (e.g., reproduction, reference toxicants)

General Testing Issues

Test lengths/endpoints
Organism age
Water Renewal (volumes, frequency, method of renewal)
Physical Test System (sediment volume, etc.)
Test condition and design (chambers, lighting, etc.)
Interpretation of sediment variables (e.g., organic carbon, particle size) on test results
Feeds/feeding regimes
QA/QC for acceptable tests

Table 1. Survey respondents for sediment test organisms.

Laboratory	*H. azteca*	*C. tentans/ C. riparius*	*L. variegatus**
Department of Fisheries & Oceans	x		
Environment Canada	x	x	
EPA-Duluth	x	x	x
EPA Region 1	x	x	
EPA Region 8	x		
EPA Newtown	x	x	
EVS Consultants	x	x	
Maryland Department of Environment	x		
Miami University	x		
Michigan State	x	x	x
NFCRC-Athens	x	x	
NFCRC-Columbia	x	x	x
NOAA-Ann Arbor	x	x	x
Old Dominion	x		
State of Washington	x		
University of Mississippi		x	
University of Wisconsin-Superior	x	x	x
Wright State	x	x	

a Two responses were on two different species.

Note: EPA region 6, Dallas, Texas, and the FDA-Center for Veterinary Medicine, Maryland, responded to survey but did not have organisms in culture.

APPENDIX D:

BIBLIOGRAPHIES FOR FRESHWATER TEST SPECIES

Group: C:\REF\CHIRSCH1.GRP
Temporary group for searching
Sorted by: Authors, Year, Title
Using Format: LIMNOL OCEANOGR
Current Search: term=chir
Last Search run on 9 Sep 1992, at 11:18
Last modified on 9 Sep 1992, at 12:53

Listed with Format LIMNOL OCEANOGR

Listing Created 9 Sep 1992, at 12:55

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