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CONSIDERATIONS IN SELECTING BIOASSAY ORGANISMS FOR DETERMINING THE POTENTIAL ENVIRONMENTAL IMPACT OF DREDGED MATERIAL

by

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A list of factors was developed to aid in the selection of test species for bioassay and bioaccumulation potential studies with dredged material. The list was compiled after interviews with personnel involved in dredged material testing from the Corps of Engineers, the Environmental Protection Agency, private consulting firms, and universities.

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20. ABSTRACT (Continued)

The factors to be considered for the selection of test species included whether the organism was indigenous to the disposal site or closely related to an indigenous species; was available through collecting or purchasing; had a toxicological data base; could be maintained and cultured in the laboratory; was useful in acute, chronic, and bioaccumulation tests; was ecologically and economically important; had a wide geographic distribution, and was compatible with other test species in the test containers.

For the selection of test species to determine bioaccumulation potential in the laboratory, the same factors were used with the additional considerations that the organism be large enough to provide sufficient tissue for chemical analyses and that they survive the 10-day exposure period.

The factors were developed as an aid in selecting test organisms and were not meant to be any more than a formal organization of considerations that should be used. The regulatory personnel and scientific investigators must exercise sound technical judgment in using the factors, together with the information available for their particular operation in selecting the test organisms.

Based on a literature review, a 10-day exposure period was sufficient time to qualitatively determine if most chemical contaminants would be accumulated from dredged material by test organisms. This length of exposure did not produce a definitive bioaccumulation study, but did allow a determination of the comparative uptake of contaminants and was a useful tool in evaluating the potential environmental impact of dredged material.

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SUMMARY

Bioassays are required as one criterion to aid in the evaluation of the potential environmental impact of open-water disposal of dredged material into the ocean. One of the problems encountered in the use of bioassays is the appropriateness of the test organisms recommended for the bioassays. Bioaccumulation potential of chemical constituents associated with dredged material is also a criterion used to evaluate the acceptability of open-water disposal of dredged material. In addition to the appropriateness of the test species being used to determine bioaccumulation potential, the validity of a 10-day exposure as a sufficient duration has been questioned.

The objective of the work described in this report was to develop a rationale to aid Corps of Engineer (CE) Districts, Environmental Protection Agency (EPA) Regions, and private consultants in the selection of appropriate test species for bioassays and bioaccumulation potential studies of dredged material for the particular set of circumstances that are applicable to their dredging and disposal operation. The validity of a 10-day exposure to determine bioaccumulation potential was also discussed.

The objective was accomplished by: interviews with CE, EPA, private consultants, and university personnel who were involved in dredged material testing; review of reports

submitted to the CE on dredged material testing; and review of the literature in scientific journals on the results of dredged material and complex waste bioassays.

As a result of the literature review and discussions, a list of selection factors was developed to aid in the selection of test species. The list must be adapted and modified by the parties involved in the testing program to meet their specific needs. Examples are presented to demonstrate the use of the selection factors. However, it was stressed that the scientific expertise of the regulatory and scientific personnel involved, and information available for a particular dredging and disposal operation, must play vital roles in the final selection.

The list of selection factors was as follows:

> The organism is found at the disposal site, or
>
> A closely related organism is found at the disposal site.
>
> The organism is readily available through field collecting or purchasing.
>
> A toxicological data base exists for the organisms:
> - General data base.
> - Dredged material data base.
>
> Response to the same toxicant is reproducible.
>
> The organism can be maintained in a healthy condition in the laboratory.
>
> The organism can be cultured and will reproduce under laboratory conditions providing sensitive life stages and sizes for testing.

The organism can be used in the major types of bioassays:
- Acute.
- Chronic.
- Bioaccumulation.

The organism occurs over a wide geographic area.

The organism is economically important.

The organism is ecologically important.

The organism is compatible with other test species.

Each factor is assigned a numerical rating and the candidate species are rated and compared to each other for each factor and then given a final score. The species with the highest score is considered the prime candidate for testing. Final scores that differ by only a few points are not considered significant and the sound technical judgment of the scientific investigators and regulatory personnel is very important in making the final selection.

The list of selection factors also applies to organisms being considered for testing the bioaccumulation potential in the laboratory. The organisms must also be of sufficient biomass to provide enough tissue for analysis of the chemical constituents of interest and must survive the 10-day exposure period.

The literature review showed that a 10-day exposure period to dredged material is a sufficient length of time to qualitatively determine if most contaminants will be taken up by the test species. While it would not be a definitive bioaccumulation study, the test as presently used does provide a useful tool in evaluating dredged material contaminants by providing information on comparative uptake of contaminants. It can be used as one of the considerations in evaluating the potential environmental impacts of dredged material disposal.

PREFACE

This study was conducted under Contract No. DACW39-78-C-0093, dated 29 September 1978 between EG&G, International, Inc., Bionomics Operation, and the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. This report is a summary of the work accomplished as a part of the WES Environmental Impacts and Criteria Development (EICD) Project. The EICD was sponsored by the Office, Chief of Engineers, U.S. Army, and monitored by the Environmental Laboratory (EL), WES. This study was also under the sponsorship of the WES Dredging Operations and Technical Support Program (DOTS).

The principal investigators were Dr. Peter J. Shuba, Technical Coordinator, Bionomics Marine Research Laboratory (BMRL), Dr. Sam R. Petrocelli, Manager of Toxicology, Bionomics Aquatic Toxicology Laboratory (BATL), and Robert E. Bentley, Species Projects, BATL. Dr. Ken Macek, BATL, and Mr. Rod Parrish, BMRL, reviewed the manuscript and made suggestions that improved the report. The authors are especially indebted to Ms. Jane Lewis for her assistance in preparation of this report.

This study was conducted under the supervision of Dr. Richard K. Peddicord, EICD, EL; and under the general supervision of Dr. Robert M. Engler, Contract Manager, EL; Mr. Charles C. Calhoun, DOTS Program Manager; and Dr. John Harrison, Chief, EL.

Commanders and Directors of WES during the conduct of this study and the preparation and publication of this report were COL John L. Cannon, CE, COL Nelson P. Conover, CE, and COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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CONVERSION FACTORS, U. S. CUSTOMARY TO METRIC (SI) UNITS OF MEASUREMENT

U. S. customary units of measurement used in this report can be converted to metric (SI) units as follows:

Multiply	By	To Obtain
fathoms	1.8288	metres
feet	0.3048	metres
miles (U. S. statute)	1.609347	kilometres

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CONSIDERATIONS IN SELECTING BIOASSAY ORGANISMS FOR DETERMINING THE POTENTIAL ENVIRONMENTAL IMPACT OF DREDGED MATERIAL

PART I: INTRODUCTION

Background

1. Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972,[1] (Public Law 92-532, hereafter referred to as the "Act") requires the Secretary of the Army to apply criteria or objectives given in Section 102 of the Act before issuing a permit for the transport of dredged material for purposes of ocean disposal. The Federal Register[2] of 11 January 1977 contains the criteria for implementation of the Act and requires bioassays for the technical evaluation of the dredged material. In July 1977, the U.S. Environmental Protection Agency (EPA) and the U.S. Army Corps of Engineers (CE) jointly published an Implementation Manual[3] (hereafter referred to as the "EPA/CE Manual") to provide guidance in conducting the required bioassays. Included in the EPA/CE Manual are lists of suggested organisms for use in the bioassays.

2. Since the publication of the EPA/CE Manual many CE Districts have had contractors use the procedures to evaluate dredged material, thus providing large-scale evaluation of the procedures. In addition, research has been

conducted by universities, governmental agencies, and private consultants that could improve the methods given in the EPA/CE Manual. A great deal has been learned about toxicity testing of complex wastes in general and dredged material in particular since the publication of the EPA/CE Manual. A major concern of all users is the suitability of the recommended groups of test organisms in all situations and the selection of appropriate species for the bioassays. The selection of appropriate species for laboratory determination of bioaccumulation potential has also become an important consideration because attempts to find a sufficient number of the same species at disposal sites have been largely unsuccessful. Selection of appropriate test species is very important because the data derived from the laboratory studies are used in decision making and are applied to "real-world" situations.

3. Among the concerns expressed by personnel involved in testing dredged material and application of the results is the fact that the list of organisms present in the EPA/CE Manual was not applicable to all geographical areas of the U.S. Some of the species are not found on all coasts, many of the organisms are not found at ocean disposal sites because their natural habitat is in estuaries, and the recommended test organisms are not normally found on or located in the types of fine-grained sediments that are dredged from harbors and navigation canals.

Objective

4. The objective of the work described in this report was to develop a rationale to aid the CE Districts, EPA Regions, and private consultants in the selection of appropriate test species for bioassays and bioaccumulation potential studies of dredged material for the particular set of circumstances that are applicable to their dredging and disposal operation. A list of selection factors was developed, but it must be stressed that the list is only intended as an aid in the decision making process. The final decision should consider the factors but should also be based on the sound judgment of the investigators, especially with respect to appropriate site-specific factors.

Scope

5. The objective was accomplished by reviewing the Act and *Federal Register* for legal considerations. The scientific literature was reviewed for discussions of tests conducted with dredged material and, where applicable, of tests of complex wastes other than dredged material. Corps of Engineer reports of the results of dredged material tests were examined. Of prime interest in reviewing the literature were the organisms tested, the rationale for selecting the organisms, and the suitability of the organisms as indicators of potential toxicity and bioaccumulation. Telephone conversations and interviews were conducted with EPA and CE personnel and contractors in private industry

and universities who have performed studies with dredged material or other complex wastes.

6. The study centered on the selection of marine organisms for testing dredged material because final criteria governing ocean dumping have been published in the Federal Register and an Implementation Manual describing test methods is available. Regulations and criteria related to the discharge of dredged material into navigable waters are currently being promulgated as required under Section 404 of the Federal Water Pollution Act Amendments of 1977 (Public Law 92-500). When published they may require bioassay and bioaccumulation studies similar to those required for ocean disposal. Therefore, when suitable examples were found for tests conducted with freshwater organisms, they were discussed in relation to suitability for dredged material testing. The literature review was by no means exhaustive. It was meant to present some of the more common test organisms being used in bioassays of dredged material and other complex wastes and the reasons that they were selected by the laboratory investigators. Detailed technical data were not summarized. Only general information which would be of interest to an investigator who may be considering using a particular organism for testing was discussed.

PART II: LITERATURE REVIEW AND PERSONAL INTERVIEWS

General Guidance

Public Law 92-532 and Federal Register

7. Public Law 92-532 does not specifically mention bioassays to evaluate the pollution potential of dredged material. Section 103 of the Act states that the Secretary of the Army must apply the factors given in Section 102 of that Act before issuing a permit for the transportation of dredged material for ocean disposal. The Secretary must determine from the use of the factors that the disposal will not "unreasonably degrade or endanger human health, welfare, or amenities, or the marine environment, ecological systems, or economic potentialities."

8. Section 102 of the Act is the basis for the requirement in the criteria for biological testing. It requires that, in reviewing applications of permits for ocean disposal, the EPA Administrator must consider (among many other things) the possible effects on human health, plankton, fish, shellfish, wildlife, and marine ecosystems in terms of productivity, stability, diversity, population dynamics of species and communities, and the persistence and permanence of the effects of disposal. Since to field test many of the factors listed in Section 102 of the Act may entail monitoring after disposal has occurred,

the intent seems to be to use laboratory bioassays to predict the potential problems of ocean disposal of wastes prior to the disposal operations.

9. The criteria published in the Federal Register[2] of 11 January 1977 require that bioassays be performed on materials proposed for ocean dumping. Section 227.4 states that EPA must determine that disposal of any waste under consideration will produce no unacceptable adverse effects on human health or the marine ecosystem and no significant damage to resources within the marine environment. According to Section 227.6, the potential for undesirable effects is determined by using appropriate bioassays of the liquid phase, suspended particulate phase, and solid phase of the wastes. Bioassays of the liquid phase and suspended particulate phase, as well as bioaccumulation from the suspended particulate phase, should be conducted with "appropriate sensitive marine organisms." Bioassays and bioaccumulation from the solid phase should be determined using "appropriate sensitive benthic marine organisms." (The three phases of dredged material are defined in Section 227.32.)

10. Appropriate sensitive marine organisms are defined in Section 227.27 of the Register as at least one species of phytoplankton or zooplankton, a crustacean

or mollusk, and a fish. Appropriate sensitive benthic marine organisms are defined as at least one species representing a filter-feeding, a deposit-feeding, and a burrowing species.

11. These requirements are very broad and there should be no difficulty complying with them. The word "appropriate" implies, among other things, that the normal habitat of the species could be impacted by the phase of dredged material in which it is to be tested. For the liquid and suspended particulate phases, the test organisms should be found in the water column all or most of the time since that is where these phases would exert the greatest effect. For the solid phase, appropriate organisms would be those that spend most of their time in or on sediment. Determination of "sensitivity" should be based on information available from the scientific literature and expert scientific opinion. Appropriately sensitive can also mean that the organism is neither so hardy it will not respond to testing nor so delicate that it cannot be maintained in the laboratory, but has an appropriate sensitivity for testing.

12. The United States is a signatory to the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (The London Dumping Convention). The provisions of the Convention require the issuance of

a permit before the ocean disposal of wastes and include lists (annexes) identifying various groups of materials and suggestions to be taken into consideration before a permit is issued. The Convention annexes list some biological considerations which can be assessed by using laboratory bioassays and bioaccumulation studies. The Convention influences U.S. domestic legislation on ocean disposal of all materials.

Testing manuals

13. The Act[1] and Federal Register[2] clearly require biological evaluation, particularly bioassays and bio-accumulation potential studies, to evaluate waste material proposed for ocean disposal. However, guidance is given only in very general terms and the Federal Register specifies that more detailed and specific procedures are to be supplied by EPA, and, in the case of dredged material, by EPA and the CE. For dredged material, EPA and CE headquarters established a technical committee composed of scientists from EPA and CE laboratories. They prepared and published the EPA/CE Manual[3] for use as guidance in determining the potential ecological impact of ocean disposal of dredged material.

14. The EPA/CE Manual contains sections on the collection and preparation of dredged material samples, gives chemical analyses of the liquid phase and

tissue from bioaccumulation studies, and lists methods for calculating initial mixing. Detailed methods are discussed for conducting bioassays using the liquid, suspended particulate, and solid phases of dredged material. Included in the methods are lists of suggested bioassay organisms. The manual suggests the following considerations* for the selection of bioassay organisms:

a. The test species should be collected from an area adjacent to the disposal site having similar water quality and sediment characteristics, but out of the influence of disposal operations.

b. The test species should be the same or closely related to the predominant species found at the disposal site during the season of the proposed operation.

15. When collection of organisms near the disposal site is not feasible, bioassay species may be selected from lists provided in the EPA/CE Manual. Selection should be made such that:

a. The organisms are related phylogenetically, or ecologically, or both, to the dominant species found at the disposal site.

b. Commercially important organisms that occur at the disposal site should be considered.

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*The lists of considerations in this report are not presented in any special order of priority or importance. They are simply summaries of the reasons presented by the respective authors for recommending or selecting the organisms in their publications. Each factor does not necessarily apply to all of the suggested organisms.

c. A mysid shrimp, *Neomysis* or *Mysidopsis*, should be included as an internal standard for comparison of different tests.

d. Juvenile forms should be used because of greater sensitivity.

16. Other documents have been published that provide the investigator with many of the basic requirements for conducting bioassays with aquatic organisms. Methods for acute bioassays with fish, macroinvertebrates, and amphibians were published by EPA[5], and in addition to general procedures, provides a list of recommended test species for freshwater, marine, and estuarine environments. The organisms were suggested for the following reasons:

a. Availability.

b. Importance.

c. Past use.

d. Ease of handling in the laboratory.

e. An effort to encourage uniformity and develop a great deal of information on a few species rather than a small amount of information on a wide variety of organisms.

17. An EPA procedures manual[6] for use in determining the acute toxicity of effluents to aquatic organisms gives a list of test species that were selected based on the following considerations:

a. Sensitive species indigenous to the receiving water.

b. Readily available.

c. Commercially or recreationally important.

d. Sensitive to most toxicants.

18. The EPA has a bioassay procedures manual[7] for regulating the ocean disposal permit program (for substances other than dredged material) that gives detailed test procedures for each test organism suggested in the manual. The writers of the procedures suggested using organisms indigenous to the disposal site when possible in addition to those recommended in the manual. The organisms were selected based on the following considerations:

a. Culturable in the lab.

b. Sensitive to many toxicants.

c. Large toxicological data base.

d. Adaptable to life-cycle tests.

19. Procedures for collecting, handling, and conducting toxicity tests with a large number of different groups of organisms have been published in Standard Methods for the Examination of Water and Wastewater.[8] The organisms recommended were selected for the following reasons:

a. Sensitive to many materials and environmental conditions.

b. Wide geographic distribution.

c. Abundant.

d. Available within a practical size range throughout the year.

e. Recreationally important.

f. Economically important.

g. Ecologically important.

h. Culture methods available for rearing including nutritional and environmental requirements.

i. Amenable to life-cycle testing.

20. Preliminary guidance for premanufacture notification of chemical substances has been published[9] by EPA. For the determination of ecological effects, a list of saltwater and freshwater invertebrates, as well as freshwater fish, is given. The animals were recommended because they are:

a. Representative of an ecologically important group in terms of taxonomy, trophic level, or niche.

b. In a position in the food chain leading to man or other important organisms.

c. Available.

d. Easily maintained in the laboratory.

e. Amenable to laboratory testing.

f. Characterized adequately taxonomically, physiologically, and genetically as to their role in natural environments.

Bioassay Results

Dredged material bioassays conducted for CE Districts under the Ocean Dumping Act

21. The following discussion is based on information provided by personnel from CE District Offices. While a large number of bioassays have been conducted with dredged material, at the time of this wr ing only

a few reports had been finalized and were available for review. However, a great deal of the completed work was discussed during personal visits and telephone conversations. The species tested, rationale for their selection, and their suitability as test organisms will be discussed in this section of the report.

22. San Francisco District. Bioassays of the liquid, suspended particulate, and solid phases have been completed for the San Francisco District with sediment collected from Moss Landing Harbor.[10] Test species for the liquid and suspended particulate phases were zoea larvae of the crab *Cancer magister* for the zooplankton requirement, *Macoma nasuta* for the mollusc requirement, and *Cymatogaster aggregata* for the fish requirement. For the solid phase, the shrimp *Crangon franciscorum* (deposit feeder), the polychaete *Nephtys californiensis* (burrower), and the clam *Macoma nasuta* (filter feeder) were tested. The fish, molluscs, and polychaetes were collected by the investigators from areas near their laboratory, not at the disposal site. The shrimp were purchased, and the crab zoea were obtained from a laboratory near the testing facility.

23. The authors of the report gave the following bases for selecting their test species:

a. Met the legal requirements set forth in the *Federal Register*.

b. Found in the vicinity of the disposal site.

c. Dominant in local assemblages.

d. Economically important.

e. Available in sufficient numbers, at a suitable life stage and size range.

f. Could be collected and maintained in the laboratory.

g. Sensitive to a variety of test materials.

h. Had appropriate life span of species; larval stage of sufficient duration for acclimating and testing.

24. Two field surveys were conducted at the proposed disposal site and it was determined that there were insufficient numbers of animals to determine bioaccumulation in the field. Therefore, a laboratory study was conducted in which the bivalve *Macoma nasuta* was exposed to the sediment for 10 days. In addition to the considerations used for species selection in the bioassays, animals had to be large enough to yield enough tissue for chemical analyses of the chemical constituents of interest.

25. Survival of fish and bivalves was high in the liquid phase prepared from the three sediment collecting stations. Crab zoea exhibited the greatest sensitivity although 18 percent mortality in the reference water was higher than the 10 percent recommended in the EPA/CE Manual. The fish and bivalves had excellent survival in the suspended particulate phase from the three stations and the reference site water. Again, crab zoea were the most sensitive of the three species tested, with 100 percent

mortality occurring in Station 1 suspended particulate phase after 48 hr of exposure.

26. Mortality of the shrimp and polychaetes in the reference site sediment for both solid phase tests was greater than the recommended 10 percent, but the results were consistent between the two tests, indicating that the mortality was caused by toxicity of the test materials. Statistically significant mortality of the polychaetes occurred in sediment from Stations 1 and 2 when compared to the reference site sediment. The bivalves had excellent survival in all treatments and provided enough tissue for analyses of bioaccumulation potential. No significant accumulation of copper (Cu), mercury (Hg), cadmium (Cd), zinc (Zn), lead (Pb), petroleum hydrocarbons, or organohalogens occurred.

27. The copepod *Acartia tonsa*, the mysid shrimp *Metamysidopsis elongata* and *Acanthomysis macropsis*, and the speckled sanddab *Citharichtys stigmaeus* were used as test organisms in the liquid and suspended particulate phases of sediments collected at the Naval Station, San Diego, Calif.[11] For the solid phase bioassays, the mysid shrimp *M. elongata* and *A. macropsis*, the polychaete *Neanthes arenaceodentata*, and the bivalve *M. nasuta* were used. Laboratory bioaccumulation tests were conducted with *M. nasuta*.

28. The reasons for selecting the species were:

- *a*. A. *tonsa* and *C*. *stigmaeus* are common to the California coast and are considered representative zooplankton and fish species, respectively.

- *b*. N. *arenaceodentata* resembles polychaetes known to be present at the disposal site, has a large toxicological data base, and is well characterized physiologically.

- *c*. M. *nasuta* is a bivalve found in waters as deep as 50 meters (m)--important because disposal sites off the California coast are usually 100 to 250 fathoms* deep.

- *d*. A. *macropsis* is taxonomically similar to *Mysidopsis* sp., which is suggested in the EPA/CE Manual as an internal standard for use in all testing programs with dredged material.

- *e*. M. *elongata* is a common organism in southern California, can be maintained in the laboratory, and is taxonomically related to *Mysidopsis* sp.

29. Two complete sets of bioassays were conducted. In the first test, the liquid and suspended particulate phase bioassay had unacceptably high reference mortality for the three test species. The solid phase bioassay had high reference sediment mortality of clams and mysids, but polychaete survival was excellent (99 percent). In the second set of bioassays, survival of fish and myside in the reference sediment was 97 percent for both, but copepod survival was only 23 percent. In the second solid phase bioassay, clam survival was 93 percent, while polychaetes and mysids had only 52 percent and 41 percent, respectively.

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* A table of factors for converting U. S. customary units of measurement to metric (SI) is presented on page vii.

30. The clam M. nasuta did not bioaccumulate significant concentrations of Cu, Hg, Cd, or Chromium (Cr) during the 10-day exposure to the test sediment. The metals were at least one order of magnitude higher in the test sediments than in the reference sediment.

31. Jacksonville District. In a summary letter report to the Jacksonville District,[12] the test organisms used for testing sediment collected from Cape Canaveral Harbor, Fla, and San Juan Harbor, Puerto Rico, were listed. For the liquid phase, the copepods *Acartia* and *Eurytemora*, the shrimp *Mysidopsis* and *Palaemonetes*, and the fish *Fundulus* were used. *Acartia*, *Mysidopsis*, *Palaemonetes*, and *Fundulus* or *Cyprinodon* were used in tests with the suspended particulate phase. The solid phase was tested using the clam *Mercenaria mercenaria*, the shrimp *Mysidopsis* and *Palaemonetes*, and the burrowing amphipod *Haustorius*. Bioaccumulation potential was determined in the laboratory by exposing *M. mercenaria* to the test sediments for 10 days.

32. All animals were collected from the east coast of Florida for use in the bioassays. Selection of species was based on availability for collection of animals found on the suggested list in the EPA/CE Implementation Manual. Data are not presented in the letter report, so evaluation of the suitability of animals was not possible. However, the letter report implied that the animals were suitable

for testing with good control survival.

Dredged material bioassays conducted for CE Districts under the Clear Water Act

33. Final regulations for the evaluation of dredged material proposed for discharge into freshwater and estuarine environments have not been promulgated. When these regulations are finalized, guidance will be available for regulating the discharge of dredged materials into navigable waters as required by Section 404 of Public Law 92-500, Federal Water Pollution Control Act Amendments of 1977.[4] However, bioassays with sediments from freshwater environments have been conducted and are discussed below in the context of species selection rationale and success.

34. Prater and Hoke[13] tested 15 sediments using the water flea *Daphnia magna*, mayfly larvae *Hexagenia limbata*, and fathead minnow *Pimephales promelas*. Thirteen of the sediments were collected from Ashtabula Harbor, Ohio, and two sediments used as reference sediments were from outside the harbor. The species were selected for the following reasons:

a. *H. limbata* larvae burrow into sediment and a great deal is known about their sensitivity to various toxicants.

b. *D. magna* is planktonic, can be cultured in the lab, and has a large toxicological data base.[14]

35. A water recycling bioassay apparatus was used in the 96-hr tests that allowed exposure of the mayfly larvae to the sediment while the fish and water flea

were exposed to recirculating water that continuously flowed over the sediment surface. Based on mortality of the organisms, the sediments were either classified as non-polluted (less than 10 percent mortality), moderately polluted (greater than 10 percent, but less than 50 percent mortality), and heavily polluted (greater than 50 percent mortality). The test organisms were considered to be suitable by the authors for the studies and different degrees of toxicity were observed with the sediments.

Dredged material and complex waste bioassays from scientific journals

36. This section reviews the literature on bioassays conducted with water extracts of sediments, whole sediment, and complex wastes by methods other than those in the EPA/CE Manual.

37. South Carolina. DeCoursey and Vernberg[15] conducted bioassays on water samples collected during dredging and disposal operations from three dredging locations in the Cooper River near Charleston Harbor, S.C. At each of the three locations, water was collected from the dredging site, approximately 65 m downstream from the dredging site, and from the diked disposal weir of the upland disposal site. The salinity was different at each of the three study locations and different organisms were selected for each location. *Daphnia pulex* juveniles were tested with samples from location 1 (0 parts per thousand [ppt] salinity),

grass shrimp larvae (Palaemonetes pugio) at location 2 (8 to 15 ppt salinity), and polychaete larvae (Polydora) at location 3 (10 to 25 ppt salinity). Juvenile and larval stages were selected because these stages are generally the most sensitive in the life cycle.

38. The parameters used to measure effects were mortality, metabolic measurements using respirometry, and swimming rates. In the mortality tests, survival of the three species was acceptable in the controls at all sampling periods. Mortality of the three species was greatest in weir water from the three study locations. Metabolic rates and swimming rates were decreased by all test waters in which the animals survived compared to the rates in controls.

39. Hoss et al.[16] used seven species of fish to assess the seawater-extractable components of sediment from Charleston Habor, S.C. Larval stages of Atlantic menhaden (Brevoortia tyrannus), pinfish (Lagodon rhomboides), flounder (Paralichthys dentatus, P. albigutta, and P. lethostigma), spot (Leiostomus xanthurus), and Atlantic croaker (Micropogon undulatus) were collected from estuaries near Beaufort, N.C. The animals were selected because they are known to spend part of their life in estuarine areas that are influenced by dredging and disposal operations and because they could be collected and held in the laboratory until testing began.

40. Five hundred grams of sediment was mixed with 1 liter (ℓ) of filtered seawater, shaken for 2 hr, and settled overnight. The resultant supernatant was used as the toxicant. All species of fish survived well in the controls during the test periods which ranged from 96 hr to 14 days. The results demonstrated that some species were more sensitive than others to sediment testing with pinfish being the most sensitive of the species tested.

41. Chesapeake Bay. Auld and Schubel[17] determined the effects of suspended sediment on the early life stages of six fish species. They exposed eggs and larvae of the blueback herring (*Alosa aestivalis*), the alewife (*Alosa pseudoharengus*), the American shad (*Alosa sapidissima*), yellow perch (*Perca flavescens*), white perch (*Morone americana*), and striped bass (*Morone saxatilis*) to suspended sediment concentrations ranging from 25 to 1,000 milligrams (mg) per ℓ for 1 to 14 days. The parameters measured were hatching success for eggs and survival of larvae. The sediment used was fine-grained, natural sediment collected from a shipping channel near Annapolis, Md. The organisms were selected because they spawn in estuarine areas which are periodically influenced by dredging and disposal operations.

42. There was no effect on hatching success of eggs of any species except white perch and striped bass, which

showed effects only in the 1,000 mg per ℓ test concentrations. Larvae were generally less tolerant to the suspended sediment than were the eggs of the same species, with American shad larvae being affected at 100 mg per ℓ.

43. California. Emerson[18] used the benthic polychaetes Ophyrotrocha labronica (adults) and Capitella capitata (larvae) to test seawater extracts of sediment from four sampling stations in Los Angeles Harbor, Calif. The animals were selected for the following reasons:

a. They could be collected from Los Angeles Harbor sediment.

b. They could be obtained from a nearby university.

c. They could be maintained in the laboratory and culture methods were available.

d. Their life histories were documented and larvae of C. capitata could be obtained in the laboratory from adult populations.

e. Previous toxicological data existed for C. capitata.

44. Sediment extracts were prepared using sediment and seawater in ratios of 1:100, 1:10, 1:4, and 1:2. The resulting extracts were not filtered or centrifuged prior to testing. Control survival was acceptable in all controls for both species. No mortality occurred in any of the treatments containing adult O. labronica after 96 hr of exposure. After 28 days, adult O. labronica exposed to one of the sediment extracts produced fewer offspring in

the highest test concentrations, but produced more offspring in the 1:100 extract than in the control. Solutions prepared from the four stations produced mortality among *C. capitata* larvae during 96-hr exposures, but in all cases mortality was less than 50 percent.

45. McConaugha[19] used the planktonic copepod *Acartia tonsa* and the epibenthic copepod *Tisbe* sp. to determine the toxic effects of heavy metals released from sediment collected from 13 stations in Los Angeles Harbor, Calif. The organisms were selected because both animals are indigenous to Los Angeles Harbor with *A. tonsa* comprising approximately 50 percent of the harbor plankton. *Tisbe* was originally collected from Los Angeles Harbor and cultured in the laboratory.

46. Sediment and unfiltered seawater were mixed in a ratio of 1:4, shaken for 30 min, and allowed to settle for 1 hr. The supernatant was filtered through 0.45-μm pore size filters before being used in the toxicity tests. Survival of *Tisbe* sp. was significantly lower than control survival in only one test solution after 96 hr. Survival of *A. tonsa* was significantly lower than the control in seven test solutions. However, control mortality was high (in some tests greater than 50 percent), creating questions about attributing test mortality to sediment toxicity.

47. Great Lakes region. Prater and Anderson[14,20] used larvae of the mayfly *Hexagenia limbata*, the water flea *Daphnia magna*, and the isopod *Asellus communis* in bioassays with sediment from Otter Creek, Ohio. In addition to these organisms, they used fathead minnows (*Pimephales promelas*) as test organisms for sediment from Duluth and Superior Harbor Basins, Minn. The selection of organisms was based on the considerations previously given for Prater and Hoke.[13] The recycling bioassay apparatus described for that study was used. The authors defined the degree of pollution based on mortality of the test species and made an attept to correlate bulk chemical analysis of the sediment to percent mortality as previously described.[12]

48. The investigators could not quantitatively correlate the mortality data with the bulk concentration of chemical constituents found in the sediments. Their conclusions on the degree of pollution of the sediments were made using only the results of the bioassays and the publication clearly indicated the utility of bioassays. In some of the bioassays mortality was high, but the authors did not attribute the toxicity to the concentration of any one constituent in the sediment. The possibility of synergistic effects between contaminants was discussed, but could not be predicted from the chemical data. The

authors used sediments as references that were out of the influence of known discharges or had been chemically analyzed and were shown to be free of the chemical pollutants of interest. Mortality was less than 10 percent for most of the animals in the controls. Of the animals tested, D. magna was generally the most sensitive.

49. Gannon and Beeton[21] used the burrowing amphipod Pontoporeia affinis in studies of sediment samples from nine Great Lakes Harbors. The tests, called "Benthos Viability and Sediment Selectivity Tests," measured survival and preference of adults for sediment types, respectively. They selected the amphipod because it was indigenous to many of the areas where disposal would occur and could be field collected. The animals were collected 4.5 km offshore in Lake Michigan from a depth of 35 m.

50. The results demonstrated that the amphipods preferred certain sediments over others, but no correlations between chemical or physical characteristics of the sediment and selection by the organisms were found. It was interesting to note that P. affinis selected sediments collected from open-water areas containing high proportions of sand. The organisms used in the study were collected from an open-water area where the sediments had a high percentage of sand. It is possible that the organisms chose sediments to which they were accustomed and, under

other conditions, could adapt to different sediment types. The authors pointed out that it was possible that certain sediments were not selected because they did not contain suitable nutrients for P. affinis, such as a native bacterial population which these organisms prefer. Interpretation of the 24- and 48-hr benthos viability (mortality) studies was complicated by the lack of dissolved oxygen measurements. As stated by the authors, the possibility existed that the high oxygen demand of some sediments may have caused the death of test organisms rather than any toxic materials that may have been present in the sediments.

51. Oregon. Swartz, DeBen, and Cole[22] used five species of benthic macroinvertebrates in solid phase bioassays of sediment collected from eight areas throughout the United States. The methods used by these authors made a substantial contribution to the procedures for solid phase bioassays that appear in the EPA/CE Manual. The organisms tested were the pelecypods Prothothaca stamina and Macoma inquinata, the polychaete Glycinde picta, the burrowing amphipod Paraphoxus epistomus, and one of the four cumaceans Diastylus alaskensis, Lamprops quadriplicata, Diastylopsis dawsoni, or Cyclaspis sp. Availability was a prime consideration in selection of the species and these species could be collected in sufficient numbers near the laboratory. Also, the organisms

live buried in the sediment and, therefore, were ecologically suitable for evaluating the effects of the solid phase of dredged material.

52. The control sediment was a sand collected from the same area from which the test organisms were collected (Yaquina Bay, Oreg.). The authors found that tne test species were not affected by burial of up to 30 millimeters of control sediment, nor by particle size of materials tested. Their preliminary tests on the suitability of each species illustrate an important point. Whenever an organism is a new candidate for solid phase bioassays, the physical effects of the sediment should be determined by using the approach described by Swartz, DeBen, and Cole.[22] Protothaca stamina was not affected by any of the test sediments. Glycinde picta and M. inquinata had significantly less survival in 2 of the 17 test sediments compared to control survival. Paraphoxus epistomus was the most sensitive animal tested showing significant mortality in 16 of the test sediments. As a result of the tests, the authors recommended the use of infaunal amphipods, especially P. epistomus, when conducting solid phase tests because of their sensitivity. Protothaca stamina was recommended as an indicator of bioaccumulation potential since they survived the 10-day test period and could provide a reasonable amount of tissue for chemical analysis.

53. New York. The marine copepods *Calanus finmarchicus*, *Temora longicornus*, and *Pseudocalanus* sp. were used in bioassays with acid-iron waste by Grice, Weibe, and Hoagland.[23] The organisms were collected from Block Island Sound, N.Y., and were indigenous to the disposal site. Dilution of the acid waste was made with filtered seawater and the organisms were exposed for 24 and 48 hr to determine toxicity and up to 18 days to measure the effects on reproduction and survival of the offspring. The organisms were selected because they:

a. Were available through collection.

b. Were indigenous to the disposal site.

c. Could be held in the laboratory for extended periods.

d. Had early life stages available for testing.

54. At concentrations of the acid waste with pH values of 6.5 or less in unbuffered seawater, significant mortality of the three species occurred among adults. The authors pointed out that the concentrations producing the toxic pH values existed for less than 3 minutes in the field and the mortality was not indicative of what might happen in the disposal area. Further, copepods exposed to toxic concentrations of the waste in buffered seawater showed no mortality at those concentrations which produced mortality at lower pH values. *Calanus finmarchicus* was transferred through acid-waste dilutions with pH values and time periods that simulated the time-concentration exposures expected at the

disposal area. Under those conditions, no mortality occurred. Reproduction of *T. longicornis* was inhibited in the test solutions used for the long-term studies, but the authors emphasized that the concentrations used would not persist in the field for 18 days. Control mortality was zero in the 24- and 48-hr exposures, except for one *T. longicornis* test where 20 percent mortality occurred in the control.

55. *Various U.S. locations*. Lee et al.[24] used the water flea *Daphnia magna* and the saltwater grass shrimp *Palaemonetes pugio* to conduct bioassays using the liquid phase of sediment from various locations. *Daphnia magna* was selected for testing because:

- *a*. A large toxicological data base exists.
- *b*. It is generally more sensitive than fish to chemicals.
- *c*. It is a representative zooplankton.
- *d*. It can be cultured in the laboratory.

Palaemonetes pugio was selected because it can be laboratory cultured and its sensitivity to a wide variety of chemicals is known.

56. Liquid phases were prepared by mixing water and various percentages of sediment (5, 10, 15, and 20 percent) and purging the mixtures with compressed air. The 10 percent liquid phase prepared from Bridgeport Harbor, Conn.,

sediment had a toxic effect on *P. pugio* in 96-hr tests, but liquid phases from Ashtabula Harbor, Ohio, and Corpus Christi Harbor, Texas, sediments had little or no toxicity toward *D. magna* and *P. pugio*, respectively. Control survival was greater than 90 percent in all tests. Manganese was released from all sediments tested. They conducted 96-hr static bioassays of dissolved manganese at concentrations representative of those released from the sediments with *D. magna* and *P. pugio*.. No adverse effects with either organism were observed at any of the concentrations tested.

57. Shuba, Tatem, and Carroll[25] conducted bioassays with the liquid, suspended particulate, and solid phases of dredged material from various locations throughout the U.S. with a variety of freshwater and saltwater organisms. Their objective was to develop biological test methods to assess the pollution potential of dredged material disposal in open water. Based on initial results, they presented a suggested list of organisms that warranted further research.

58. For saltwater tests, they suggested the copepod *Acartia tonsa*, the epibenthic isopod *Sphaeroma quadridentatum*, the mysid shrimp *Mysidopsis* sp., the burrowing amphipod *Parahaustorius* sp., and larvae of the estuarine grass shrimp *Palaemonetes* sp. For bioaccumulation potential, they suggested *Rangia cuneata* and *Mercenaria mercenaria*.

For freshwater tests, they suggested the grass shrimp (*Palaemonetes kadiakensis*), the fingernail clam (*Musculium* sp.), and the zooplankter *Daphnia pulex*. The asiatic clam (*Corbicula manilensis*) was recommended as suitable for determination of bioaccumulation potential from freshwater sediments.

59. Organisms were selected that:

a. Had a toxicological data base.

b. Could be collected in sufficient numbers for testing.

c. Could be transported long distances, particularly saltwater organisms, in a healthy condition.

d. Could be maintained in the laboratory for reasonable periods of time.

60. Buikema, Lee, and Cairns[26] selected *Daphnia pulex* as a test organism to evaluate the potential environmental impact of oil refinery wastes. In attempting to develop a standard method, they formulated an arbitrary reference mixture (ARM) that contained six compounds commonly found in oil refinery effluents and known to be toxic to many forms of aquatic life. After initial screening tests using 15 species of freshwater invertebrates and three species of freshwater fish, they selected *Daphnia pulex* for further testing because it:

a. Was the most sensitive species tested.

b. Was inexpensive and easy to maintain in the laboratory culture.

c. Was ecologically important as a fish food organism.

d. Is distributed over the entire North American continent in a wide range of habitats.

The ARM was toxic to *D. pulex* after 48 hours of exposure. The method using *D. pulex* as the test species proved to be reproducible among different laboratories and was relatively easy to conduct in a short period of time.

61. Hall, Buikema, and Cairns[27] tested saltwater organisms by using the ARM and, after initial screening, found two genera of grass shrimp, *Palaemonetes* and *Hippolyte*, and the pinfish *Lagodon rhomboides* to be the most sensitive of the species tested. The grass shrimp *P. pugio* was selected for additional testing based on:

a. Sensitivity.

b. Commercial availability at a low cost.

c. Could be maintained in the laboratory.

d. Wide geographic distribution.

e. Availability in different size and age groups.

Results of the tests with *P. pugio* demonstrated that salinity and photoperiod had no effect on the toxicity of ARM. Grass shrimp were more sensitive to the toxicant at higher temperatures, but only during the first 48 hr of exposure. Larval grass shrimp were more sensitive than adults, but the effect was not observed after 24 hr of exposure. Grass shrimp collected from six geographic regions showed no significant difference in sensitivity to the ARM, and there was no significant difference among three species: *P. pugio*, *P. intermedius*, and *P. vulgaris*, when tested in mixed populations.

Bioaccumulation Results

62. The problems encountered and the rationale used in species selection for bioassays of dredged material also apply to selection of species for use in determination of bioaccumulation potential. In addition, test organisms must be of sufficient biomass to provide enough tissue for analyses of the chemical constituents of interest. Great difficulty has been encountered in collecting enough animals of the same species for the determination of field bioaccumulation as described in the EPA/CE Manual. Therefore, animals used in the 10-day solid phase bioassay are being used for the determination of bioaccumulation potential. The validity of a 10-day bioaccumulation study has been seriously questioned by some investigators. This portion of the report will address the topic of the validity of a 10-day bioaccumulation study and other pertinent problems associated with laboratory studies involving bioaccumulation of contaminants from complex wastes.

63. Pesch and Morgan[28] demonstrated that both the toxicity and bioaccumulation of Cu from seawater decreased in the presence of sand. Adult male polychaetes (*Neanthes arenaceodentata*) were exposed to Cu in a continuous-flow

seawater system with and without the presence of sand. The 28-day LC50's (the concentration of Cu causing mortality of 50 percent of the test animals after 28 days of exposure) were 0.044 mg/ℓ without sand and 0.10 mg/ℓ with sand. Animals exposed to 0.04 and 0.06 mg Cu/ℓ in the presence of sand had significantly lower Cu concentrations than those exposed to 0.04 mg Cu/ℓ in the absence of sand.

64. Pesch[29] demonstrated that the toxicity of Cu to *N. arenaceodentata* was significantly decreased in the presence of sand, mud, and a sand and mud mixture compared to toxicity in seawater alone. The times required to reach 50 percent mortality were 7.8, 36.5, 50, and 54.5 days for seawater, sand, mud, and the sand and mud mixture, respectively. All sediment treatments received 0.10 mg Cu per ℓ of seawater. The concentration of Cu in the polychaetes was dependent on the duration of exposure; the longer the exposure period, the higher the Cu concentration. Interestingly, the worms exposed to the mud had the highest mean Cu concentration in spite of the fact that the time to 50 percent mortality was longer than in the animals exposed to sand or seawater.

65. Jackim, Morrison, and Steele[30] using radioactive Cd, demonstrated a decrease of Cd accumulation in the marine bivalves *Mya arenaria* and *Mulinia lateralis* in the presence of sediment. The animals were exposed to

the radioactive metal in recirculating, filtered natural seawater for 7 days. Two types of sediments were used, a beach sand containing 0.2 percent organic matter and a bottom sediment collected from Narragansett Bay, R.I., that contained 1.5 percent organic matter. The Cd concentration in *M. arenaria* in the presence of beach sand was 23 percent less than that accumulated from seawater. The Cd uptake by clams in bottom sediment was 53 percent of that of clams in the absence of sediment. The same trend in reduced uptake in the presence of sediment occurred with *M. lateralis* although the differences were not statistically significant.

66. Roberts and Maguire[31] used natural assemblages of meiofauna associated with sediment to determine the toxic effects of Pb. They collected surface (top 5 centimeters [cm]) and subsurface (7 to 15 cm deep) sand, added Pb to the laboratory seawater, and followed the development of the communities that inhabited the sediments. Although there was a great deal of variation in the data, the authors made some interesting observations and conclusions. Chemical analyses of the interstitial waters showed Pb was not detectable at any of the concentrations added after the initial (0 hr) samples. Based on the meiofaunal communities that developed in the different sediment samples, subsurface sand was more effective in removing Pb from the water and the metal was less toxic in the presence of high concentra-

tions of organics and fine particulates than in coarse sand or clean water.

67. The polychaete Nereis diversicolor was exposed to sediments contaminated with radioactive plutonium (Pu) and americium (Am).[32] Two sources of sediment were used in the uptake studies: Bikini Atoll sediment contaminated as a result of the testing of nuclear devices, and sediment from the Irish Sea contaminated as a result of the reprocessing of nuclear fuel. Animals were collected from uncontaminated sediment and allowed to depurate the contents of their intestinal tract for 24 hr. They were then placed in the test sediments using a flowing seawater system.

68. After 40 days of exposure, the polychaetes contained approximately 0.5 percent of the concentration of the elements in the sediments. The uptake of Pu was comparable in both sediments and it was preferentially taken up over Am. Uptake increased throughout the 40-day exposure to Irish Sea sediment, but the concentration of Pu was significantly greater than the initial concentration after 5 days. Generally, the uptake of Pu and Am was significantly greater after 5 and 15 days of exposure, respectively, compared to the control (0 hr). Based on the results of their study and published data, they presented calculations and estimated that 83 percent of the Pu found in the worms came from the interstitial water.

69. Louma and Jenne[33] studied the uptake of Cd in the clam *Macoma balthica*. The animals were collected from San Francisco Bay and acclimated to laboratory conditions for five days. To determine whether sediment or water was the major source of the metal, the clams were placed into the sediment and in dialysis bags above the sediment in static aquaria systems. By comparing the total amount of radioactive Cd taken up to the clams in the dialysis bags, they were able to estimate the amount of the metal derived from the sediment.

70. In experiments using San Francisco Bay sediment labeled with radioactive Cd, it was determined that statistically significant uptake occurred from the sediment after 8 days of exposure, although total uptake continued for 42 days. Very little uptake occurred when ^{109}Cd was organically bound to detritus particles or to iron oxide particles coated with organics. However, significant uptake from uncoated iron oxide particles did occur. The results indicated that organically bound Cd may be unavailable to deposit feeding clams, such as *Macoma balthica*, and that sediments with high organic content may have less bioavailable metals even though the total concentration may be higher than some other types of sediments. In experiments where uptake did occur, the tissue concentration never exceeded 15 percent of the sediment concentration.

71. Ayling[34] collected oysters and sediment from the same sampling stations of the Tamar River in Tasmania and examined the concentrations of Cd, Cu, and Cr in the samples. Attempts were made to find correlations between the concentration in the animals and in the sediment. The resulting correlations were poor and it was determined that chemical analysis of sediments could not be used to predict areas for safe oyster harvesting because it could not estimate the concentration of contaminants in oyster tissue.

72. The study further demonstrated that bioaccumulation factors of 100,000 to 300,000 based upon uptake from seawater are misleading because in the study it was found that only 10 to 40 times the concentrations of these metals existed in oysters compared to sediments. Some trends were also observed in the study. Copper and chromium reached a maximum concentration in oyster tissue dependent on the size of the oysters, not the concentration in the sediment. Lead concentrations in oysters were generally dependent on the amount in the sediment, but there was a great deal of variability in the data. Zinc and cadmium concentrations were higher in oysters collected from sediments with high metal concentrations and size of the animals did not seem to influence uptake of these metals.

73. Bryan and Hummerstone[35] investigated the concentrations of Zn, Pb, manganese (Mn), iron (Fe), and

Cu in the burrowing polychaete *Nereis diversicolor* compared to those found in the animals' environment. The polychaetes were collected from the sediment and put in 50 percent seawater with a sand substrate for a 7-day depuration period. The tissue was then analyzed for the metals and compared to the concentrations found in the sediments.

74. The trends were that Cu concentrations in the worms were related to the Cu concentrations in the sediments, but Zn, Pb, Mn, and Fe were not. The concentration of Zn was "amazingly" constant regardless of the sediment concentrations. The authors discussed the possibilities that the organisms may have regulatory mechanisms for some metals such as Zn, but not Cu, and that the total concentration of a metal in a sediment may not be related to the amount that is biologically available. In sediment transfer experiments, animals from sediment with low Cu concentrations were transferred to sediment with high Cu concentrations; after 28 days of exposure, the Cu concentration in the animals had increased by a factor of eight. After 10 days of exposure, the polychaetes had accumulated approximately 80 percent of the maximum Cu concentration. Also, animals from sediment with high Cu concentrations transferred to lower Cu concentration sediments had no loss of the metal after 7 weeks. Polychaetes exposed to Cu in solution demonstrated that animals collected from

high Cu-containing sediments were less sensitive than animals collected from low Cu-containing sediments.

75. Renfro[36] used the marine polychaetes, <u>Nereis diversicolor</u> and <u>Hermione hystrix</u>, to determine the amount of Zn that would be taken up by the animals and what effect the animals would have on the release of the metal from the sediment. Zinc was added to the sediments as ^{65}Zn and experiments were carried out in flowing seawater systems. After 5 days of exposure to the radioactive sediment, <u>N. diversicolor</u> had accumulated 0.2 percent of the ^{65}Zn added to the sediment, and after 10 days of exposure had accumulated 50 to 70 percent of the concentration that polychaetes exposed for 48 days had accumulated. When transferred to nonradioactive sediment, the polychaetes lost approximately 30 percent of the radioactivity in 3 days. In the absence of the animals, 1 to 3 percent of the ^{65}Zn was desorbed from the sediments in 18 days and 3 to 9 percent in 30 days. The burrowing activity of <u>N. diversicolor</u> increased the loss of the metal by three to seven times that which occurred in their absence.

76. Fowler <u>et al.</u>[37] conducted uptake studies from seawater and sediment using polychlorinated biphenyls (PCB) and the polychaete <u>N. diversicolor</u>. To determine the uptake from water, the animals were exposed in flowing

seawater containing a PCB mixture. In the sediment uptake study, the animals were exposed to a fine-grained sediment that had been thoroughly mixed with a PCB mixture until chemical analyses indicated homogeneity.

77. A steady-state concentration was reached in 2 weeks by polychaetes exposed to PCB in seawater with the concentration factor being 800. Uptake from the sediment was concentration dependent; steady-state concentrations were reached in about 2 months at the lower PCB concentrations, but uptake continued for 3 months at the higher concentration. After 10 days of exposure to sediment with PCB, the animals had accumulated significant concentration of PCB in all exposure concentrations. The concentration factor for sediment uptake was three to four.

78. Elder, Fowler, and Polikarpov[38] exposed _N. diversicolor_ to sediment spiked to a final concentration of 0.65 ppm of a PCB mixture. The control was the same sediment without the addition of PCB. Seven hundred and fifty polychaetes were added to the control and to the contaminated sediment and analyzed after various periods of exposure. The worms reached equilibrium with the concentration of PCB in sediment after 40 to 60 days. However, uptake occurred after only 5 days of exposure and the difference between the control and experimental tanks was statistically significant.

79. Langston[39] adsorbed the PCB Aroclor® 1242, 1254, and 1260 onto alumina particles and exposed the cockle *Cerastoderma edule* and the tellin *Macoma balthica* for periods up to 40 days. The concentrations of total PCB as well as homologs of different chlorine content were determined in the animal tissues.

80. After 10 days exposure, *C. edule* had accumulated 31, 34, and 23 percent of the concentration of Aroclor 1242, 1254, and 1260, respectively, that were accumulated in the experiment. *Macoma balthica* accumulated 20, 11, and 53 percent of the final concentration of Aroclor 1242, 1254, and 1260, respectively, after 10 days exposure. Generally, isomers containing five and six chlorine atoms were accumulated to the highest concentration and more rapidly than isomers with more or less chlorine atoms in both test animals. The concentration of individual chlorinated biphenyl compounds increased as the number of chlorine atoms increased to five atoms, then decreased as the number of chlorine atoms increased beyond five atoms.

81. In a study to determine the depuration rates of PCB, Langston[40] exposed *C. edule* to alumina particles coated with Aroclor 1242, 1254, 1260, or individual chlorinated biphenyls for 10 days followed by a 21-day depuration period. The animal accumulated significant amounts of PCB mixtures and individual compounds after

10 days of exposure. After 21 days of depuration, *C. edule* had significantly decreased the concentration of 1242, but not 1254 and 1260. *Cerastoderma edule* significantly reduced the concentration of chlorinated biphenyls containing five or less chlorine atoms in 21 days, and it was found that the configuration of the molecules influenced elimination. Molecules with orthosubstituted chlorine atoms were eliminated faster than those with chlorine atoms in the metaposition or paraposition.

82. Fossato and Canzonier[41] exposed the mussel *Mytilus edulis* to suspensions of kaolin previously mixed with diesel fuel to coat the particles with petroleum hydrocarbons. Three seasonal experiments involving uptake and depuration were conducted during different times of the year. Uptake was immediate and rapid when the mussels were exposed to the kaolin-coated particles in the three exposures. In the June to September exposure, after 10 days the mussels had accumulated about 50 percent of the 40-day concentration. After the first 10 days of exposure in the November to January and March to June experiments, about 90 percent of the maximum uptake concentration had been reached. Depuration was also immediate and rapid in the three experiments. In addition to the obvious variable of animals collected at different times of the year, the water temperature and salinity, acclimation

period, test concentration, and exposure period varied among the three tests.

83. Neff, Foster, and Slowey[42] conducted laboratory bioaccumulation studies using marsh clams *Rangia cuneata*, estuarine grass shrimp *Palaemonetes pugio*, freshwater grass shrimp *P. kadiakensis*, polychaetes *N. arenaceodentata*, and the tubificid worms *Tubifex* sp. All animals were collected from their natural environments and availability of sufficient numbers for testing was an important criterion for species selection. The animals were exposed to sediments from Texas City and Corpus Christi, Tex. (saltwater), and Ashtabula, Ohio (freshwater), for 6 weeks. Bioaccumulation was determined at salinities of 0, 15, or 30 ppt using appropriate species. Tissues were analyzed for Cd, Cr, Cu, Fe, Mn, Pb, Zn, Hg, vanadium (V), and nickel (Ni).

84. Significant uptake occurred 36 times (26.5 percent) out of the possible 136 combinations tested. In 13 (9 percent) of the 136 combinations, the concentration of metal was higher in the control tissue than in the animals exposed to test sediments. In many cases where significant uptake of metals occurred, there was a significant difference between the exposed and control tissues within the first 10 days of exposure.

85. Among the conclusions of the authors were that:

a. No correlation existed between uptake of the metals and total concentration in the sediment.

b. No correlation existed between accumulation and the chemical form of the metal based on selective extraction of the sediment.

c. Salinity significantly affected metal uptake.

d. There was variation in uptake among the test species of the same metal.

e. There was variation in uptake of the different metals within the same species.

f. There was a difference in uptake in the species collected at different seasons of the year.

g. Polychaetes and grass shrimp accumulated metals more times than did clams.

86. The authors recommended using two or more species in each bioaccumulation study and stated that a 3- to 4-week exposure period would be ample time to determine bioaccumulation potential of most chemical constituents from sediment.

Interviews Concerning Species Selection for Bioassays

87. The authors contacted persons from regulatory agencies such as the CE and EPA, industrial and academic contractors who were conducting bioassays according to the EPA/CE Implementation Manual, and persons for whom testing was being conducted, such as port authorities, to ascertain their viewpoints on the species suggested in the Manual, other species they have tested or might suggest, and their rationale for selecting test species. After initial telephone conversations, those persons found to be significantly involved in dredged material testing were visited for personal interviews.

88. Among the questions asked were:

- a. What were their opinions of species presently suggested in the Manual?
- b. What species were they presently using?
- c. What successes or problems had been encountered with the test species?
- d. Have they experienced problems in handling, culturing, or maintaining the organisms?
- e. Have they had any problems with control animals (e.g., poor survival)?

89. Responses tended to follow geographic division of the United States and will be reported in that manner except in the cases where many respondents from different geographical areas expressed the same concern.

Pacific coast

90. Several investigators on the west coast thought that the lists of recommended species were developed largely for the east coast of the United States. They were concerned with the degree of restriction placed on them and said that at least two lists, one for the east coast and one for the west coast, should be developed. They thought that there should be a further breakdown to include northern Pacific species and southern Pacific species. Many investigators suggested that any list of specific test organisms would be too restrictive for their purposes and wanted lists completely open-ended, which would allow for the selection of the most appropriate species for a particular set of bioassays.

Several persons suggested that the selection process should be based on a numerical rating system.

91. The concept of using organisms that inhabit shorelines to evaluate the toxicity of dredged material disposed of at deep water sites, and, given this, whether the information obtained with these species is readily extrapolable to offshore species, was questioned. On the other hand, the requirement for the collection of species from the disposal site could create many problems since the majority of disposal sites on the west coast range in depth from 100 to 250 fathoms (600 to 1,500 ft). Many investigators stated that it was highly unlikely that adequate numbers of any organism could be found at such depths to conduct tests according to the EPA/CE Manual. In addition, the collection of benthic organisms at depths of 250 fathoms would be economically exorbitant. They suggested that organisms which can be cultured with relative ease should be used as test species. Suggested deep-water forms that are presently in culture in southern California are the deep-water shrimps *Acanthomysis macropsis* and *Metamysidopsis elongata*. The ability to culture and maintain many of these organisms through a complete life cycle should be seriously considered since certain investigators are of the opinion that partial or full life

cycle testing affords much more complete information on the effects of pollutants in the dredged material.

92. In the northern California, Oregon, and Washington areas, in addition to the concerns listed above, some investigators stated that it was very important to determine the susceptibility of commercial and sport fishes and shellfishes. To this extent, juvenile salmonids (*Oncorhynchus* sp.) and crab (*Cancer magister*) larvae were mentioned by several persons as being representative of sizable fisheries. The salmonids in particular are known to be quite sensitive to many toxicants and may therefore be an excellent choice. Relative ecological abundance should also be considered in selection. The shallow-water clam *Mulinia* sp. was suggested in this context. Other frequently mentioned species included the shiner perch *Cymatogaster aggregata*, the amphipod *Corophium* sp., the polychaetes *Nephthys* sp. and *Glycinde* sp., and the bent-nose clam *Macoma nasuta*. The use of infaunal species of amphipods, such as *Paraphoxus epistomas*, was strongly recommended because of its sensitivity in dredged material testing based on published literature. Additional species mentioned by individuals, because of their sensitivity and availability, included the shrimp *Crangon franciscorum*, the razor clam *Silque* sp., the deepwater shrimp *Pandalus jordanii*, the zooplankter *Acartia* sp., the bivalve *Protothaca stamina*, and larvae of the oyster

Crassostrea gigas.

93. In the southern California area, investigators were concerned that test temperatures of 10°C were recommended in the EPA/CE Manual when most of the species indigenous to this area are more appropriately tested at 19°C. Some of the species frequently mentioned included the zooplankter Acartia tonsa, the polychaete Neanthes arenaceodentata, and the deepwater shrimps Acanthomysis macropsis and Metamysidopsis elongata. Some investigators suggested using the speckled sanddab Citharichthys stigmaeus because it is a very common fish in California waters while others felt this species was not appropriate, in part because of its susceptibility to ciliated parasites. Other species suggested were the bent-nosed clam Macoma nasuta, the broken-back shrimp Heptacarpus pictus, the nearshore shrimps Crangon nigromaculata and Acanthomysis sculpta, the bivalves Crassostrea gigas, Mytilus edulis, and M. californianus and larvae of the crab Cancer magister. These organisms were suggested because they are ecologically abundant, readily available, and many can be cultured or maintained in the laboratory. Before bioassays can be conducted in EPA Region IX, the organisms to be used must be agreed upon by EPA, CE, National Marine Fisheries, National Fish and Wildlife Service, and California Fish and Game Commission.

Atlantic coast

94. The investigators along the Atlantic coast had parallel concerns to their counterparts on the Pacific coast. They were concerned about species availability, cost of acquisition of test species, and being able to culture or maintain animals in the laboratory. The people in this area have adhered very closely to the species listed in the EPA/CE Manual and have had reasonable success in dredged material toxicity tests. However, in solid phase tests mysid shrimps (*Mysidopsis bahia* and others) have consistently died in the supposedly uncontaminated reference sediments for many investigators, raising strong objections on the part of these persons. The regulatory agencies consider *M. bahia* an excellent species and think that it should be used, citing its sensitivity to chemicals and the ease with which it is cultured and maintained in the laboratory as important considerations.

95. Region II of EPA (New York) has been requiring specific animals for dredged material bioassays conducted under its jurisdiction. As a result of the requirements, a great deal of uniformity exists with the animals that have been used by various contractors. The New York District, CE, has published a guidance manual[43] for use in conducting dredged material tests which reflects the EPA requirements. The District Manual requires the marine diatom *Skeletonema*

costatum, Atlantic silversides Menidia menidia, and mysid shrimp Mysidopsis sp. for the liquid and suspended particulate phases, and the hardshell clam Mercenaria mercenaria, grass shrimp Palaemonetes sp., and the polychaetes Neanthes sp. or Nereis sp., for the solid phase tests. These organisms parallel the requirements of Region II EPA which suggest Acartia sp. instead of S. costatum in some cases, and for dredged material tests with sediment from Puerto Rico, require Menidia beryllina in place of M. menidia and the use of the white sea urchin Tripneustes esculentus. The organisms are recommended because they have been used in a large number of bioassays of industrial wastes and have an extensive toxicological data base, can be maintained in the laboratory, and are readily available through collection or commercial sources.

96. In addition to the organisms listed above, the shrimp Crangon and Neomysis, the zooplankton Pseudocalanus, and the bivalves Nucula and Yolida, have been used in the New England area. The organisms were selected because of availability and ease of maintenance in the laboratory.

97. In the Charleston, S.C., area, liquid and suspended particulate phase tests have been conducted with Menidia sp., Palaemonetes pugio, Mysidopsis bigelowi, and larvae of the crab Callinectes. Solid phase tests have utilized M. mercenaria, Neomysis sp., and the burrowing amphipod Haustorius sp. The organisms were selected because

they could be found at the disposal site, had a toxicological data base, were available through field collection or commercial sources, and had larval stages available for testing.

Gulf of Mexico

98. Organisms used for bioassays of dredged material from areas in the northern Gulf of Mexico were selected from the lists provided in the EPA/CE Manual because they could be collected from estuarine areas and maintained in the laboratory until used for testing or were being cultured in the contractors laboratory. Animals that had a large toxicological data base were given significant consideration. A few of the organisms were found at disposal sites during field surveys.

99. Organisms used along the northern Gulf Coast were *Mysidopsis almyra* and *M. bahia*; *Menidia beryllina*; sheepshead minnows *Cyprinodon variegatus*; the polychaetes *Nereis* sp., *Neanthes* sp., and *Diopatra* sp.; *Palaemonetes* sp.; marsh clam *Rangia cuneata*; oysters *Crassostrea virginica*; hard-shell clam *M. mercenaria*; and burrowing amphipods *Parahaustorius* sp. Many contractors have had problems using mysid shrimp in solid phase tests. This has been particularly evident in tests with dredged material and reference sediments from the Louisiana and Texas coasts. These areas have bottom sediments that are composed of very fine particulate matter.

In solid phase tests, the fine particulates remained suspended during most, if not the entire, 10-day period. Mysid shrimp are apparently sensitive to exposure to the suspended particulates resulting in mortality that was not attributable to toxicants associated with the test materials.

Freshwater testing

100. The topic of freshwater species presents a completely different set of circumstances. No formal procedures exist for the testing of dredged material in fresh water, and some investigators are using the general guidance provided in a draft of Section 404(b)(1) guidelines, while others are formulating their own procedures. The investigators contacted felt that it was important to use species indigenous to the disposal area. Those species which have been utilized in freshwater tests include the burrowing mayfly *Hexagenia limbata*, the gammarid amphipods *Asellus communis* and *Gammarus* sp., the water flea *Daphnia magna*, the fathead minnow *Pimephales promelas*, the channel catfish *Ictalurus punctatus*, and the mussels *Amblema* sp. and *Fusarcia* sp. These species are either relatively easy to culture or may be collected and maintained in the laboratory for reasonable periods of time. A species which is presently being considered for bioaccumulation testing is the yellow perch *Perca flavescens*. While this species is widely

distributed, a current limitation on its use is that the availability of the hatchery-reared perch is severely limited.

PART III: DISCUSSION AND CONCLUSIONS

101. Historically, the most important considerations used in the selection of saltwater organisms for toxicological testing were the ability of the investigator to obtain sufficient numbers of the organism and to maintain them in good physical condition for reasonable periods of time in the laboratory. Organisms which satisfied these considerations included species which were relatively hardy in their ability to withstand the rigors of manipulation by the investigator. Classical examples of such species include the brine shrimp *Artemia salina*, grass shrimp *Palaemonetes vulgaris* and *P. pugio*, hard shell clam *Mercenaria mercenaria*, American oysters *Crassostrea virginica*, and the mummichog *Fundulus heteroclitus*. While not a primary consideration in species selection, a species which also had some economic importance was favored.

102. Once a data base that would permit a comparison of the relative sensitivities of these species to chemical exposure had been developed, sensitivity became a more heavily weighted consideration for selecting a particular species

from those available for testing. That is, the most sensitive species with which the investigator could reasonably work in the laboratory were used to provide an estimate of the effects of chemicals on aquatic species with the implicit understanding that a few species might exist that were still more sensitive to chemical exposure than were the standard test organisms. Some examples of these so-called "sensitive" species are the calanoid copepod Acartia tonsa, penaeid shrimp Penaeus sp., and Atlantic silversides Menidia menidia. The standard test organisms were selected as representative species to be used as indicators of the environmental effects of a specific perturbation.

103. More recently, the ability to maintain a test population in the laboratory for a complete reproductive cycle has become a very desirable factor for the selection of a test species since this ability permits an assessment of the effects of chronic exposure to a chemical on the reproductive capacity, growth, and development of the organism. Examples of saltwater species which satisfy this consideration are mysid shrimp Mysidopsis bahia and sheepshead minnows Cyprinodon variegatus.

104. As described in the section summarizing the results of meetings with regulatory personnel and contractors who have conducted dredged material bioassays according to the methods described in the EPA/CE Manual, one of the major problems encountered in the performance of these tests is the

selection of appropriate, sensitive marine organisms for use in these bioassays. Criticisms of species recommended in Tables D1 and F1 in the EPA/CE Manual include the fact that many of these species are not present at dredged material disposal sites, or, if present, cannot be collected in sufficient numbers for testing or maintained in good condition in the laboratory. Many of the species listed in the tables are east or Gulf coast species and not common to the Pacific coast. Furthermore, the mysid shrimp appear to be unsuitable for this type of testing because of problems unrelated to the pollutant content of dredged material to be tested.

105. The considerations for species selection most often used in the literature reviewed in this report and by people interviewed concerning dredged material testing included availability, toxicological data base and sensitivity, presence of the species or closely related species at the disposal site, and ability to maintain or culture the organisms in the laboratory, all of which were significant considerations. The factors for selecting test organisms have been tabulated along with the number of times they appeared in the present report (Table 1). They are not arranged in order of importance, but are simply a summary of the considerations used by those authors whose publications were reviewed and those people with whom discussions were

held concerning species selection. The literature review on bioassay and bioaccumulation results was by no means exhaustive, but was intended to be representative of current literature which describes the results of complex waste testing. It is interesting to note that many of the test species utilized, and the reasons for selecting them, occur repeatedly in the literature.

106. The concensus of the persons interviewed appeared to be that there should be more latitude in the selection of test species for dredged material bioassays. Most investigators prefer to work with species with which they are familiar in terms of ability to collect, maintain, and test successfully in the laboratory. Tests with these species would yield precise (reproducible) data while tests conducted with some of the recommended species have yielded unreliable data caused at least in part by the investigators' lack of understanding of the normal physiological requirements of the species.

PART IV: RECOMMENDATIONS FOR SPECIES SELECTION

107. At present, the nomination of alternative test species to regulatory personnel by investigators has met with a great deal of resistance and investigators have generally been required to choose from among the species

listed in the EPA/CE Manual. Regulatory personnel generally do not have the appropriate background regarding the toxicological response of saltwater organisms to determine whether or not the substitution of an alternative test species will adversely affect the utility of the study. Therefore, some mechanism should be established which will permit regulatory personnel to obtain a judgment from a designated standing committee of toxicologists. At a minimum this committee should be composed of one aquatic toxicologist representing the CE and one from the EPA Office of Research and Development. For maximum usefulness this committee should be kept as small as possible. Outside experts from government, academia, industry, or testing laboratories could be consulted as necessary.

108. A potential method for selection of appropriate species for dredged material testing would involve the assignment of a numerical rating to nominated species based on the degree to which each satisfied selection factors agreed upon in advance by the regulatory agencies involved. The numerical rating for each factor is totaled for each species and then compared among the various nominated species as an aid in making the final choice. A scheme of this type has been proposed by Oshida.[44] Among the factors on which Oshida rated potential test organisms were abundance and collectability, sensitivity, mobility,

reproductive potential, and size. In order to devise a scheme of this type which has relevance to dredged material testing, the authors of the present report propose the use of the following considerations based on the literature review and personal interviews related to dredged material testing. The list of considerations are not presented in order of importance and are based on the work conducted for this project and the experience of the authors. The considerations would also apply to any bioassay requirements promulgated in the future for Section 404 of Public Law 92-500.

a. Suitability of test organisms to meet the legal requirements stipulated in the 11 January 1977 Federal Register.

b. Presence of organisms (or closely related species) at the disposal site.

c. Availability (preferably year-round) of organisms through collection near the disposal site, other areas (e.g. estuaries), or through purchase.

d. Existance of a large toxicological data base; known sensitivity to a wide variety of chemicals, including dredged material, under diverse environmental conditions.

e. Reproducibility of response to the same toxicant.

f. Amenability to maintenance in the laboratory in a healthy condition for periods of acclimation and testing.

g. Amenability to culture and reproductive success in the laboratory to supply an adequate number of test organisms of most usable size and biomass and sensitive life stages (e.g. larvae and juveniles).

h. Amenability to various bioassay procedures:

  (1) Acute testing.

  (2) Chronic testing or sensitive life stage testing.

  (3) Bioconcentration testing.

i. Distribution over wide geographic region.

j. Ecological significance.

k. Economical significance and representative of a position in the food chain leading to man.

l. Existance of information on the physiology and genetic groups from different trophic levels.

m. Representative of widely differing phylogenetic groups from different trophic levels.

n. Tolerance of test species to the particle-size distribution of the control, reference, and test sediments.

o. Compatibility with the other species being tested to avoid predator-prey relationships, allow testing in as few containers as possible, and not require special precautions to be taken.

109. The use of organisms that are indigenous to the disposal site, or adjacent areas, has the distinct advantage of measuring the effect on representative organisms that may be impacted during the disposal operation, thus providing the most realistic measure of the potential environmental impact. To date, however, it has not been feasible to collect the large number of organisms required to conduct a complete set of solid phase tests from disposal site areas. It is suggested that an initial field survey be

conducted to determine what organisms occur in the area and if there are sufficient numbers for collection to be used in testing. If the organisms are not abundant, the survey will provide data on organisms that are present during the season of proposed disposal operation and will aid in the selection of alternate test species. In some cases, enough information concerning indigenous species could be obtained from published literature. The field survey will add additional expense to the project and should only be considered for the larger disposal operations.

110. Obtaining sufficient representative test organisms can be accomplished through field collection or purchase through commercial suppliers. Reish[45] pointed out the advantages of using laboratory populations of animals for toxicity tests which included:

a. Available when needed for testing.

b. Adapted to laboratory conditions, such that acclimation to test conditions such as salinity and temperature may not be necessary.

c. Diet is defined; a valuable asset if bioaccumulation is of interest.

d. Life histories and physiology of laboratory animals may be defined to a greater degree than field-selected animals and the information may contribute to long-term testing.

e. Interlaboratory comparisons are possible when all animals are from the same source.

111. Perkins[46] discussed some of the problems associated with conducting marine toxicity tests and pointed out the difficulties encountered in collecting animals from the field, transporting them to the laboratory, and acclimating them to test conditions without physiologically stressing the animals and affecting the results of the tests. He pointed out that laboratory-reared animals may have undergone genetic changes that may make their sensitivity to a toxicant quite different from the response of wild populations.

112. The investigator must have a sufficient number of animals to conduct the tests regardless of the source. Consideration should be given to the following questions:

a. Are the selected organisms "representative" of animals found near the disposal site?

b. Have the organisms been shown to be sensitive to a variety of toxicants?

Organisms of the same species that have been previously used in dredged material testing programs should be given primary consideration. Organisms that are sensitive to toxicants in solution may not exhibit the same sensitivity to the toxicant in the presence of sediment. Also, organisms must be selected that can tolerate the physical stress of exposure to sediment in an aquarium if the test is to be successful. The test should measure the effect of chemical contaminants associated with the sediment rather than decreases in dissolved oxygen concentration or temporary increases in turbidity.

113. The selection of sensitive organisms must be based on literature reviews and discussions with personnel conducting aquatic bioassays. Organisms that have been used in toxicological testing programs and are sensitive to many kinds of toxicants should be given serious consideration. When chemical data exist on major contaminants in the sediment, consideration should be given to animals that have shown a sensitivity to those contaminants present in the sediment. The results of tests with the organism should be reproducible.

114. Once sufficient organisms are obtained, they must be maintained in the laboratory until they are acclimated to the test conditions and used for testing. Organisms that can be held in the laboratory throughout their life cycle and that can reproduce under laboratory conditions are usually excellent test organisms. Early life stages can thus be obtained and tested as well as the production of various sizes of animals to meet the needs of the particular investigation. Although laboratory-reared animals have certain disadvantages previously discussed, the advantages of using them currently outweigh the disadvantages.

115. Acute toxicity tests are the most numerous types conducted, but it is desirable to test the most sensitive life stage of an animal through partial or full life cycle tests. The number of organisms currently available for chronic tests is limited, but research in the area will add to the list in the future. The duration of life span, reproductive success in the laboratory, and life stages available for testing are important considerations.

116. The present requirements for dredged material testing require acute tests and bioaccumulation potential determination. Chronic tests are not required, but organisms that can be used in chronic exposures are highly desirable in the event that the investigator wants to extend the period of exposure. Organisms with a wide geographic distribution are desirable because the results of tests with the organisms would have a wider application and could be compared to results of tests in various areas of the United States. Organisms that are economically important warrant testing because damage to their population would cause a loss of revenue to a sector of society. In theory, all organisms are ecologically important, but a knowledge of their importance to the ecosystem under investigation makes the choice of appropriate species more relevant. Compatability of different organisms is important because it could simplify

establishing the test by using fewer test containers and will help avoid predator-prey relationships and the resultant loss of test organisms. Finally, organisms that can be used in the solid phase exposure for 10 days and then be used for the determination of bioaccumulation potential are desirable.

117. Measuring bioaccumulation potential from dredged material with field-collected animals has been successful in only a few instances, and, when it has been used, only selected contaminants have been analyzed in the tissue. When all of the constituents listed in Table G1 of the EPA/CE Manual have been of interest, 10-day laboratory tests have been conducted.

118. The literature review concerning bioaccumulation indicated some of the problems and considerations that investigators must consider when conducting laboratory studies with dredged material. Bioaccumulation (and toxicity) of a particular contaminant decreases in the presence of sediment when compared to the same contaminant in seawater even when the total concentrations are equal. Generally, sediments with high organic content decrease uptake and toxicity of metals compared to the same concentrations in seawater. In most cases, the concentration of a contaminant in test animal tissue is only a fraction of the total concentration occurring in the sediment. For most contaminants, correlations between the concentration occurring in sediment

and animal tissue do not exist. Finally, the bioconcentration factors (BCF) for uptake from sediment are much lower than the BCF from seawater for a particular contaminant. Obviously, the total concentration in the sediment is not the concentration that is biologically available.

119. It is the opinion of many members of the scientific community, shared by the authors, that a 10-day test with dredged material is not sufficient for a definitive study of bioaccumulation potential, but it does provide a reasonably good qualitative estimate. Moreover, the literature review indicated that statistically significant uptake of contaminants often occurs after 10 days of exposure when test animal tissue concentrations are compared to those of animals in a reference sediment or initial concentrations. The laboratory bioaccumulation study does provide useful information on comparative uptake of contaminants and can be used as one of the considerations in evaluation of the potential environmental impacts of dredged material.

120. Laboratory studies offer some advantages that must be considered. The literature review demonstrated that uptake varied depending on the contaminant being studied, test species being used, size and age of the animals, the season in which the animals were collected, and, for some contaminants, the chemical concentration in the sediment. Other parameters known to influence uptake of chemicals include salinity, temperature, pH, and the presence

or absence of other contaminants. Variables such as salinity, temperature, and pH can be controlled and standardized in the laboratory. The results of uptake can then be compared between experimental and control animal tissues and serve as one tool in evaluation of ecological impact. Laboratory studies are also less expensive than field determinations, which is a practical consideration.

Examples for Using the Selection Factors

121. The organisms discussed in the literature review are acceptable for some, but not all, dredged material testing situations. The authors of this report agree with those persons interviewed who were of the opinion that guidance is needed not on specific test species but on the rationale for selecting the most appropriate species for the test in question in consideration of site-specific requirements. These requirements will vary geographically, seasonally, and with the sediment type to be tested. The selection factors can be used as a guide to species selection, but scientific judgment of the investigators is also extremely important.

122. A list of selection factors and a suggested rating system for species selection are given in Table 2. Organisms under consideration would be rated based on the number of criteria that apply to them. The organisms receiving the highest score would then be the prime candidate

for use in the bioassays. In some instances, the test species receiving the highest score may not be the animal that is finally selected. In the scheme presented, it is assumed that the organisms under consideration meet all of the legal requirements set forth in the Federal Register.[2] The selection factors should be weighed differently by the regulatory agencies, depending on the site-specific conditions.

123. A hypothetical case will illustrate the use of the selection factors. A dredged material disposal operation is proposed for an approved interim site off the north Pacific coast which has been used in the past. The site has not been characterized biologically and no information on indigenous species is available. Four species, A, B, C, and D, are under consideration to meet the requirement for a burrowing species for solid phase testing. Each selection factor was applied to the proposed test species with the rating for each organisms totaled and compared (Table 3).

124. Information on organisms at the disposal site was not available so factor 1 was not used. All of the organisms are available, but species B and D could be easily collected or purchased for a more reasonable price than A and C. A great deal of general toxicity data had been generated for all four species, but species B had

been used in a number of dredged material solid phase tests. The literature indicated that reproducible data were produced when the 4 species were tested with the same reference toxicant. Species A and B were easily maintained in the laboratory, but only species B could be cultured in the laboratory and had various ages and sizes available for testing. All of the species could be used for acute tests, but only species B could be used for chronic tests and species C for bioaccumulation tests. Species C was the only organism known to have wide geographic distribution. None of the organisms had any economic importance, but species B was known to be an extremely important fish food organism. None of the organisms were thought to be compatible with the other test species. Species B clearly received the highest score and would be considered the primary candidate for the burrowing species (Table 3).

125. A second example (Table 4) involves the selection of a deposit-feeding species for use in a solid phase bioassay. The dredged material is proposed for disposal in the Gulf of Mexico. It is known that species A, C, and D occur at the disposal site during some portion of their life cycle and organisms related to these species also occur at the disposal site. All organisms were readily available, but species D could be collected in large numbers with minimum effort. All of the species had general and dredged material toxicological data bases, but species B had been used in

many dredged material tests. Species B and D were known to respond to standard toxicants in a consistent manner. The four species could be maintained in the laboratory, but B and C were easier to maintain and only A could be cultured in the laboratory. All four species could be used for acute tests, but only A could be used in chronic tests. Both species B and D were acceptable for bioaccumulation studies and species D was large enough to provide enough tissue for the number of contaminants that were going to be analyzed in the bioaccumulation study. Species A, C, and D were widely distributed. Species D was economically important as well as having some ecological significance. None of the organisms would be compatible with the other test species. Species D received the highest rating and would be the species of choice (Table 4).

126. The rating scheme is subjective. In the examples presented, only 0, 5, or 10 points were given to the factors. Individual investigators may assign any numbers in the rating scheme that are appropriate. Also, the maximum score for any factor may be changed and one or more factors presented may be deleted with others added when the particular dredged material test requires such changes and the regulatory agencies are in agreement with the changes. The selection factors and ratings are suggestions to aid the laboratory investigators and regulatory agencies in organizing

the species selection process. The final selection of a test species will be made by using a series of selection factors, the scientific expertise of the regulatory and scientific personnel involved, and information available for the particular dredging and disposal operation. Although the examples presented were for the selection of test animals for solid phase testing, the selection factors and procedures would be the same if the investigator were considering different species for testing the liquid and suspended particulate phases. The species receiving the highest score may not always be the species that will be tested. For example, an organism may receive the highest score, but is not available during the season the testing program will occur. Also, when two species are separated by only a few points, either species is acceptable.

Further Recommendations

127. If species selection were based on less restrictive guidelines such as the suggested factors, species for which there are few or no data describing toxicological response may be selected for testing because of their relevance to the site in question. Under these circumstances, the regulatory agency must have some means of comparing the sensitivity of the selected species with other species for which data are available. To generate these data, the investigator would be required to conduct static acute toxicity tests with the species he has selected for testing, exposing the organisms

to several reference toxicants of different generic types. These reference toxicants should be among those for which a large data base on acute toxicity to aquatic species exists. Some examples are organochlorine insecticides (DDT, aldrin, dieldrin, endrin), organophosphate insecticides (malathion, parathion, guthion), carbamate insecticides (carbaryl), surfactants (DSS), and metals (silver, copper, and cadmium). Results of static acute tests with three of these reference toxicants would provide information describing the relative sensitivity of the selected test species.

128. The need to find additional bioassay animals exists for all test materials, including dredged material. As the science of aquatic toxicology progresses, additional test animals that will serve as representative organisms will be found. One area of research which should be investigated is the effect of dredged material on fertilization and development of fish eggs, such as the work of Auld and Schubel.[17] In general, these types of tests would be extremely relevant to assessing the potential environmental impact of dredged material because of sensitivity and economic and ecological importance of numerous fish species.

129. A criticism of the solid phase test as presented in the EPA/CE Manual is that it does not determine long-term effects of the disposal of dredged material on benthic communities. The developing community studies such

as those of Hansen[47] and Davis et al.[48] would be an excellent approach to evaluating long-term effects. In these studies, aquaria are established which contain a clean sediment and unfiltered natural seawater is continuously supplied to all aquaria. The test material (dredged material in this case) is added to the exposure aquaria, but not the controls, for a period of eight weeks or longer if appropriate. The benthic macroinvertebrates that develop in each treatment are identified and quantitated, and control and test aquaria are statistically compared. This approach could be adapted for dredged material testing by following community development in and on dredged material and an appropriate control. Tagatz, Ivey, and Lehman[49] used the community study approach to evaluate the effects of a drilling mud and determined that there were statistically significant effects on the development of macrobenthic communities caused by the presence of the drilling mud.

130. The results of bioassay and bioaccumulation studies with dredged material as presented in the EPA/CE Manual were intended to be only one of the considerations used in the evaluation of a permit application for dredging and dredged material disposal. In practice, however, the results of these tests have unfortunately been the sole determinant for denying or granting a permit. A formal hazard evaluation scheme should be developed for evaluating potential dredging and disposal projects. An excellent hazard evaluation

scheme is presented by the American Society for Testing and Materials (ASTM)[50] that could serve as an outline for the development of a scheme for dredged material. The ASTM defines hazard evaluation as "an assessment of the probability that adverse effects will result from the use or release of a substance in a specified quantity and manner." They present a tiered approach to testing and consider such factors as patterns of material usage, disposal and release, chemical and physical properties, biological properties, existing toxicological data, and estimated water concentrations. A formal hazard evaluation scheme for evaluating a dredging project would consider the results of laboratory bioassay and bioaccumulation studies as one of many factors in the overall decision-making process.

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Table 1

Summary of Reasons for the Selection of Test Organisms from the Literature Review and Personal Communications

Selection Factor	Number of Times the Factor Appeared in this Report
Available through collection or purchase.	23
Can be maintained in the laboratory.	17
Sensitive to a wide variety of toxicants and test conditions.	14
Can be cultured in the laboratory.	14
Toxicological data base.	11
Presence of species, or closely related species, at the disposal site.	11
Ecologically important.	11
Abundant and have a wide geographic distribution.	9
Different sizes and ages available including larval and juvenile stages.	8
Economically important.	7
Adaptable to life-cycle testing.	3
Recreationally important.	2

Table 2

Selection Factors and Suggested Rating System for Selection of Test Species for Use in Dredged Material Bioassays and Bioaccumulation

Selection Factor	Maximum Rating
The organism is found at the disposal site, or	10
A closely related organism is found at the disposal site.	5
The organism is readily available through field collecting or purchasing.	10
A toxicological data base exists for the organisms:	
General data base.	5
Dredged material data base.	10
Response to the same toxicant is reproducible.	5
The organism can be maintained in a healty condition in the laboratory.	10
The organism can be cultured and will reproduce under laboratory conditions providing sensitive life stages and sizes for testing.	10
The organism can be used in the major types of bioassays:	
Acute	10
Chronic	5
Bioaccumulation	10
The organism occurs over a wide geographic area.	5
The organism is economically important.	10
The organism is ecologically important.	10
The organism is compatible with other test species.	5
TOTAL	120

Table 3

Hypothetical Result Using the Selection Factors to Chose a Burrowing Species for Use in a Solid Phase Bioassay of Dredged Material from the North Pacific

Selection Factor	Rating Received per Species			
	A	B	C	D
The organism is found at the disposal site, or	0	0	0	0
A closely related organism is found at the disposal site.	0	0	0	0
The organism is readily available through field collecting or purchasing.	5	10	5	10
A toxicological data base exists for the organisms:				
General data base.	5	5	5	5
Dredged material data base.	0	10	5	0
Response to the same toxicant is reproducible.	5	5	5	5
The organism can be maintained in a healthy condition in the laboratory.	10	10	0	5
The organism can be cultured and will reproduce under laboratory conditions providing sensitive life stages and sizes for testing.	0	10	0	0
The organism can be used in the major types of bioassays:				
Acute	10	10	10	10
Chronic	0	10	0	0
Bioaccumulation	0	0	10	0
The organism occurs over a wide geographic area.	0	0	5	0
The organism is economically important.	0	0	0	0
The organism is ecologically important.	5	10	5	5
The organism is compatible witn other test species.	0	0	0	0
TOTAL	40	80	50	40

Table 4

Hypothetical Result Using the Selection Factors to Chose a Deposit-Feeding Species for Use in a Solid Phase Bioassay of Dredged Material from the Gulf of Mexico

Selection Factor	Rating Received per Species			
	A	B	C	D
The organism is found at the disposal site, or	10	0	10	10
A closely related organism is found at the disposal site.	5	0	5	5
The organism is readily available through field collecting or purchasing.	5	5	5	10
A toxicological data base exists for the organisms:				
General data base.	5	5	5	5
Dredged material data base.	5	10	5	5
Response to the same toxicant is reproducible.	0	5	0	5
The organism can be maintained in a healthy condition in the laboratory.	5	10	10	5
The organism can be cultured and will reproduce under laboratory conditions providing sensitive life stages and sizes for testing.	5	0	0	0
The organism can be used in the major types of bioassays:				
Acute	10	10	10	10
Chronic	5	0	0	0
Bioaccumulation	0	5	0	10
The organism occurs over a wide geographic area.	5	0	5	5
The organism is economically important.	0	0	5	10
The organism is ecologically important.	10	0	10	5
The organism is compatible with other test species.	0	0	0	0
TOTAL	70	50	70	85

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CONSIDERATIONS IN SELECTING BIOASSAY ORGANISMS FOR DETERMINING --ETC(U)
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APPENDIX A:

PERSONNEL CONTACTED BY TELEPHONE OR VISITS TO THEIR OFFICES TO DISCUSS SELECTION OF ANIMAL SPECIES FOR CONDUCTING DREDGED MATERIAL BIOASSAYS

1. Corps of Engineers Personnel

PERSON	OFFICE LOCATION	TELEPHONE
Armstrong, John	Seattle District, WA	206-764-3625
Bechley, Jack	Portland District, OR	503-221-6450
Bellmer, Rus	Los Angeles District, CA	213-688-5421
Bradley, Paul	Mobile District, AL	205-690-2723
Carroll, Mike	New England Division, MA	617-894-2400
Cullen, Mike	St. Louis District, MO	314-268-2130
Earhart, Glenn	Baltimore District, MD	301-962-4501
Enoch, Larry	Buffalo District, NY	716-876-5454
Frankensteen, David	Wilmington District, NC	919-343-4752
Lang, Paul	Buffalo District, NY	716-876-5454
Lawrence, James	New Orleans District, LA	504-838-2532
Maki, Mel	Portland District, OR	503-221-6450
Medina, Rick	Galveston District, TX	713-763-1211
Morrison, Steve	Charleston District, SC	803-724-4259
Moulding, Jim	Jacksonville District, FL	904-791-2286
Moulton, Bob	Portland District, OR	503-221-6450
Neidergang, Norm	Chicago District, IL	
Rasgus, Jane	North Central Division, IL	312-886-5465
Reese, Jim	Portland District, OR	503-221-6450
Slerzak, William	New York District, NY	212-264-5620
Slowenski, Tom	Chicago District, IL	
Smith, Ed	Pittsburg District, PA	412-644-6922
Snitz, Frank	Detroit District, MI	313-226-6753
Suster, John	San Francisco District, CA	415-556-5370
Wexler, Bill	Albuquerque District, NM	505-766-2781
Whiting, Paul	St. Paul District, MN	612-725-5934
Yourk, Tom	Savannah District, GA	912-233-8822

2. Environmental Protection Agency Personnel

PERSON	OFFICE LOCATION	TELEPHONE
Anderson, Peter	Region II, New York, NY	201-321-6689
Anderson, Max	Region V Laboratory, Chicago, IL	312-353-8370
Cole, Fay	Newport Field Station, OR	503-867-4031

PERSON	OFFICE LOCATION	TELEPHONE
DeBen, Wally	Newport Field Station, OR	503-867-4031
Lee, Ron	Region X, Seattle, WA	206-442-1352
Swartz, Rick	Newport Field Station, OR	503-867-4031
Vais, Chris	Region IX, San Francisco, CA	415-556-3454
Wilder, Rus	Region I, Boston, MA	617-233-5061

3. Contractors or Sponsors

PERSON	OFFICE LOCATION	TELEPHONE
Christian, Ken	David W. Taylor Naval Ship R and D Center Annapolis, MD	301-267-3535
Galloway, Ken	Soils Control Laboratory Watsonville, CA	408-724-5422
Groover, Don	NUS Corporation Houston, TX	713-488-1810
Horne, Jim	NUS Corporation Houston, TX	713-488-1810
Martin, Mike	Port of Los Angeles Los Angeles, CA	213-548-7805
McGlade, Randy	Allan Hancock Foundation Los Angeles, CA	213-741-2053
McLeod, Guy	New England Aquarium Boston, MA	617-742-8830
Oguri, Mikihiki	Allan Hancock Foundation Los Angeles, CA	213-741-2053
Prater, Bayliss	Heidelburg College Tiffin, OH	419-448-2201
Salazar, Mike	Naval Ocean System Center San Diego, CA	714-225-2559
Soule, Dorothy	Allan Hancock Foundation Los Angeles, CA	213-741-2053

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