United States Environmental Protection Agency

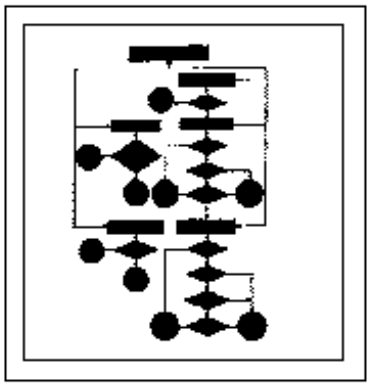
Department of The Army
U.S. Army Corps of Engineers

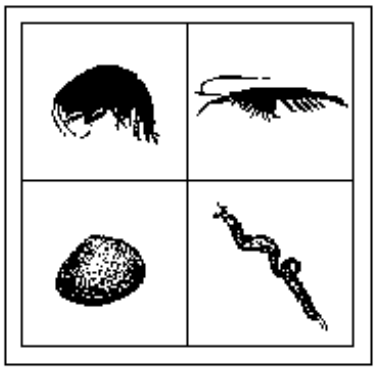
EPA 503/8-91/001
February 1991

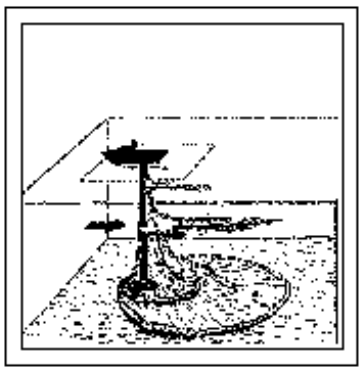
Office of Water (4504F)

Evaluation of Dredged Material Proposed for Ocean Disposal

Testing Manual







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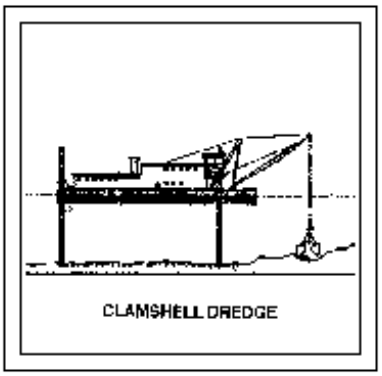

PREFACE

According to Section 103 of Public Law 92-532 (the Marine Protection, Research, and Sanctuaries Act of 1972), any proposed dumping of dredged material into ocean waters must be evaluated through the use of criteria published by the Environmental Protection Agency (EPA) in Title 40 of the Code of Federal Regulations, Parts 220-228 (40 CFR 220-228). This testing guidance manual contains procedures applicable to the evaluation of potential contaminant-related environmental impact of the ocean disposal of dredged material. It will be periodically revised and updated as warranted by advances in regulatory practice and technical understanding. This manual was approved by EPA and the United States Army Corps of Engineers (USACE) in 1991 and it replaces the July 1977 manual, *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters*, which is no longer applicable.

Review of this manual was conducted by EPA through the Marine Operations Division of the Office of Marine and Estuarine Protection and by the USACE through the Office of the Chief of Engineers and the Environmental Laboratory of the Waterways Experiment Station. Significant input on regional issues that have National relevance was received from EPA Region and USACE District staff and incorporated into the appropriate sections of this document.

Part I: General Considerations

1.0 INTRODUCTION

This manual, commonly referred to as the "Green Book," is an update of *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters* (EPA/USACE, 1977). The manual contains technical guidance for determining the suitability of dredged material for ocean disposal through chemical, physical, and biological evaluations. The technical guidance is intended for use by dredging applicants, laboratory scientists, and regulators in evaluating dredged-material compliance with the United States Ocean Dumping Regulations.

Integral to the manual is a tiered-testing procedure for evaluating compliance with the limiting permissible concentration (LPC) as defined by the ocean-dumping regulations. The procedure comprises four levels (tiers) of increasing investigative intensity that generate information to assist in making ocean-disposal decisions. Tiers I and II utilize existing or easily acquired information and apply relatively inexpensive and rapid tests to predict environmental effects. Tiers III and IV contain biological evaluations that are more intensive and require field sampling, laboratory testing, and rigorous data analysis.

This manual provides National technical guidance for use in making LPC compliance determinations for proposed discharges of dredged material; it does not provide comprehensive guidance on other factors that should be considered during the sediment-evaluation process. Decision-making, involving the evaluation of regulations and local policies, site conditions, and project-specific management actions to limit environmental impacts, is addressed in other Environmental Protection Agency (EPA)/United States Army Corps of Engineers (USACE) guidance manuals.

1.1 BACKGROUND

Section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (MPRSA), Public Law 92-532, specifies that all proposed operations involving the transportation and dumping of dredged material into ocean waters have to be evaluated to determine the potential environmental impact of such activities. This is performed by the Secretary of the Army, using criteria developed by the Administrator of the EPA. In accordance with Section 103 of the MPRSA, the USACE is the permitting authority for dredged material, subject to EPA review. Environmental evaluations have to be in accordance with applicable criteria published in Title 40, Code of Federal Regulations, Parts 220-228 (40 CFR 220-228), hereafter referred to as *the regulations.* Proposed ocean disposal of dredged material also has to comply with the permitting and dredging regulations given

in Title 33 CFR, Parts 320-330 and 335-338.

Appendix A of this manual contains a reprinting of 40 CFR Parts 220-228. However, this manual addresses only the technical requirements that apply to contaminant evaluation (see

.. 227.6 and 227.13).

One of the main purposes of Section 103 of the MPRSA is to regulate and limit adverse ecological effects of ocean dumping of dredged material. Consequently, the regulations emphasize evaluative techniques such as bioassays and bioaccumulation testing, which provide relatively direct estimates of the potential for environmental impact.

1.2 APPLICABILITY

This manual is applicable to all activities involving the transportation of dredged material for the purpose of dumping it in ocean waters outside the baseline from which the territorial sea is measured. The guidance in this manual is applicable to dredging operations conducted under permits as well as to Federal projects conducted by the USACE. In this manual, terms such as *dredging project,* etc., are used in the broadest sense to include Federal projects as well as operations conducted under permits. The procedures in this manual do not apply to activities excluded by . 220.1 of the regulations.

Although it is important to remember that the regulations are legally binding and that the guidance provided in this manual is necessarily responsive to the specific requirements of these regulations, the manual is *not* intended to carry the force of law. This document does, however, contain jointly acceptable technological approaches for evaluating the potential environmental impact of the ocean disposal of dredged material as agreed upon by EPA and the USACE.

1.3 PURPOSE AND SCOPE

This manual was developed under the direction of a joint EPA/USACE work group and provides a balance between technical state-of-the-art and routinely implementable guidance for using the evaluative procedures specified in the regulations. Guidance is included on the appropriate uses and limitations of the various procedures and on sound interpretation of the results.

This manual contains summaries and discussions of the procedures for ecological evaluation of dredged material required by the regulations, tests to implement them, definitions, sample-collection and preservation procedures, evaluative procedures, calculations, interpretive guidance, and supporting references required for the evaluation of dredged-material discharge applications in accordance with the regulations. Even so,

this manual cannot stand alone. It is imperative that the supporting references be consulted for detailed or more comprehensive guidance whenever indicated. Before any evaluations are begun, THIS MANUAL *AND ESPECIALLY THE REGULATIONS IN 40 CFR 220-228* SHOULD BE READ IN THEIR ENTIRETY, and citations and references should be consulted to obtain an understanding of the guidance that the manual provides. The technical procedures in this manual are designed only for dredged material and should not be used for any other materials unless definitive research demonstrates their applicability.

This manual contains evaluative procedures considered to be acceptable tools for regulation. As warranted through experience with this manual and the development of new procedures, sections of this manual will be updated periodically and the availability of these updates will be announced. Because this manual is National in scope, it cannot address every local concern, and cannot provide detailed guidance appropriate to every such issue. Therefore, development of more detailed implementation guidance tailoring the procedures of this manual to local needs is encouraged. It is essential to the ecological evaluation approach in the manual that detailed technical agreements on the approaches to be used for all disposal applications be developed jointly and cooperatively by the EPA Regional Administrator and the USACE DIstrict Engineer, by considering the input of involved local parties and the appropriate scientists in both agencies. Local guidance has to comply with all applicable regulations, and should be compatible with the guidance in this manual. If there is disagreement between an EPA Region and a USACE District, disputes should be resolved jointly by the headquarters of EPA and the USACE.

This manual does not address management actions that could be used to reduce impact associated with dredged-material disposal. Management actions for dredged material can include control of dump releases, disposal-site capping, submarine burial, and predisposal treatment. However, these actions are both project- and region-specific and are beyond the scope of the National guidance provided by this manual. The decision as to whether such material might be allowable for ocean disposal under the MPRSA and other applicable regulations, and the procedural steps to be followed in making this determination, are issues that are beyond the scope of this manual.

1.4 ORGANIZATION OF THIS MANUAL

This manual is organized into three parts and two appendices. Part I, General Considerations, presents the purpose and background of the manual and summarizes the Federal regulations that are relevant to dredged-material evaluation. Part II, Evaluation of Potential Environmental Impact, presents guidance on the testing and evaluation of dredged material that is proposed for ocean disposal. Sections 4.0 through 7.0 of Part II describe the components of the four tiers in the tiered-testing procedure. Part III, Data Generation, presents guidance on sampling, physical and chemical analysis, biological-effects evaluation, statistical methods, and quality assurance. Appendix A is a reprint of the ocean-dumping regulations (40 CFR 220-228) and Appendix B provides

technical guidance for using the numerical models to calculate initial mixing.

1.5 CHANGES FROM AND REVISIONS TO THE PREVIOUS MANUAL

This manual replaces the document *Ecological Evaluation of Proposed Discharges of Dredged Material into Ocean Waters*, published by EPA/USACE in 1977 (reprinted in 1978). This revised manual provides implementation guidance compatible with the 1977 Ocean Dumping Regulations (40 CFR 220-228) and reflects experience gained since 1977 with environmental regulation of the ocean disposal of dredged material. Although many changes have been made in the format and content of the manual, the general approach of providing the technical rationale of the regulations, test procedures, and interpretive guidance is the same, and this manual is consistent with the provisions of the existing regulations. The test endpoints and evaluative guidance have been refined, but the basic concepts are similar to those of the preceding manual.

The manual has been structured for better presentation of the expanded available information on environmental evaluation of dredged material. Part I is similar in content to Parts I and II of the 1977 manual, but with the addition of a Section that discusses the concepts of tiered testing and appropriate reference and control materials. Part II addresses how to evaluate potential environmental impact at each tier of evaluation, and provides guidance on how to use the results at each tier to make decisions. Part III is analogous to the appendices of the 1977 manual. It gives field and laboratory guidance for gathering data and discusses quality assurance/quality control considerations.

1.6 DEFINITIONS

The following terms are briefly defined and interpreted for purposes of this document. See Subpart G of the regulations for complete definitions of terms used in the regulations.

Acute toxicity

> Level of mortality by a group of marine organisms that have been affected by the properties of a substance, such as a contaminated sediment. The acute toxicity of a sediment is determined by quantifying the mortality of appropriately sensitive organisms that are put into contact with the sediment, under either field or laboratory conditions, for a specified period.

Bioaccumulation

> The accumulation of contaminants in the tissues of organisms through any route, including respiration, ingestion, or direct contact with contaminated sediment or water. The regulations require that bioaccumulation be

considered as part of the environmental evaluation of dredged material proposed for ocean dumping. This consideration involves predicting whether there will be a cause-and-effect relationship between an animal's presence in the area influenced by the dredged material and an environmentally important elevation of its tissue content or body burden of contaminants above that in similar animals not influenced by the disposal of the dredged material.

Constituents

Chemical substances, solids, organic matter, and organisms associated with or contained in or on dredged material.

Control sediment

A natural sediment essentially free of contaminants and compatible with the biological needs of the test organisms such that it has no discernable influence on the response being measured in the test. Test procedures are conducted with the control sediment in the same way as the reference sediment and dredged material. The purpose of the control sediment is to confirm the biological acceptability of the test conditions and to help to verify the health of the organisms during the test. Excessive mortality in the control sediment indicates a problem with the test conditions or organisms, and can invalidate the results of the corresponding dredged material test.

Disposal site

A precise geographical area within which ocean disposal of dredged material is permitted under conditions specified in permits issued under . 103 of the MPRSA. Such sites are identified by boundaries established by (1) coordinates of latitude and longitude for each corner or by (2) coordinates of latitude and longitude for the center point and a radius in nautical miles from that point. Appropriate data for latitude and for longitude should be indicated. Boundary coordinates shall be identified as precisely as is warranted by the accuracy with which the site can be located by using existing navigational aids or through the implantation of transponders, buoys, or other means of marking the site.

Dredged material

Material excavated or dredged from waters of the United States and ocean waters.

Dumping

The disposition of material subject to the exclusions of paragraph 220.2(e)

of the regulations and 33 CFR 320-330 and 335-338.

Initial mixing

That dispersion or diffusion of liquid, suspended particulate, and solid phases of dredged material that occurs within 4 h after dumping. The limiting permissible concentration (LPC) shall not be exceeded beyond the boundaries of the disposal site during initial mixing, and shall not be exceeded at any point in the marine environment after initial mixing.

Limiting permissible concentration (LPC)

The LPC for the liquid-phase concentration of dredged material in the water column is the concentration that, after allowance for initial mixing, does not exceed applicable marine water-quality criteria (WQC) or a toxicity threshold of 0.01 of the acutely toxic concentration. The LPC of the suspended particulate and solid phases is the concentration that will not cause unreasonable toxicity or bioaccumulation (see . 227.27 of the regulations for the complete definition).

Management action

Those actions that may be considered necessary to rapidly render harmless the material proposed for disposal in the marine environment (e.g., nontoxic, nonbioaccumulative).

May

May is used to mean "is allowed to"; *can* is used to mean "is able to"; and *might* is used to mean "could possibly."

Must

Must in this manual refers to requirements that have to be addressed in the context of compliance with the ocean dumping regulations.

Ocean

Those waters of the open seas lying seaward of the baseline from which the territorial sea is measured [see paragraph 220.2(c) of the regulations].

Reference sediment

A sediment, substantially free of contaminants, that is as similar as practicable to the grain size of the dredged material and the sediment at the disposal site, and that reflects the conditions that would exist in the vicinity

of the disposal site had no dredged-material disposal ever taken place, but had all other influences on sediment condition taken place. These conditions have to be met to the maximum extent possible. If it is not possible to fully meet these conditions, tests should use organisms that are not sensitive to the grain-size differences among the reference sediment, control sediment, and dredged material. The reference sediment serves as a point of comparison to identify potential effects of contaminants in the dredged material.

Regulations

Procedures and concepts published in 40 CFR 220-228 for evaluating proposals for dumping dredged material in the ocean.

Should

Should is used to state that the specified condition is recommended and ought to be met unless there are clear and definite reasons for not doing so.

Whole sediment

The sediment and interstitial waters of the proposed dredged material or reference sediment before it has undergone any processing that might alter its chemical or toxicological properties. For purposes of this manual, press-sieving to remove organisms from test sediments, homogenization of test sediments, compositing of sediment samples, and additions of small amounts of seawater to facilitate homogenizing or compositing sediments may be necessary to conducting bioassay tests. These procedures are unlikely to substantially alter chemical or toxicological properties of the respective whole sediments. Alternatively, wet sieving, elutriation, or freezing and thawing of sediments may alter chemical and/or toxicological properties, and sediment so processed should not be considered as whole sediment for bioassay purposes.

1.7 REFERENCES

EPA/USACE. 1977. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredged and Fill Material, *Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters*. Implementation Manual for Section 103 of Public Law 92-532 (Marine Protection, Research, and Sanctuaries Act of 1972). July 1977 (2nd printing April 1978). Environmental Effects Laboratory, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

2.0 OVERVIEW OF THE REGULATIONS

The potential effects of ocean disposal of dredged material on marine organisms and human uses of the ocean may range from unmeasurable to important. These effects may differ at each disposal site, and have to be evaluated on a case-by-case basis. The regulations provide the requirements for such an evaluation, with an emphasis on the direct assessment of biological impact. The permitting procedure for proposed ocean disposal of dredged material is given in Part 225 of the regulations. Part 227 puts forth the requirements that apply to dredged-material technical evaluation and contains procedural requirements for evaluating all dredged materials proposed for ocean dumping. Section 227.1 of the regulations makes some, but not all, sections of Part 227 applicable to dredged-material evaluations. This Section of the manual summarizes the major requirements for dredged-material evaluations. However, it is essential that decisions be based on a full reading and application of the regulations, and not on this summary.

2.1 PART 225: CORPS OF ENGINEERS (USACE) DREDGED-MATERIAL PERMITS

The application and authorization for ocean disposal of dredged material are outlined in Part 225. Section 225.2 establishes the informational requirements for evaluating proposed dredged-material actions, and . 225.3 describes the procedure for evaluating the economic feasibility of alternative methods or sites. The Marine Protection, Research, and Sanctuaries Act of 1972 (MPRSA) and Part 225 allow a waiver of the criteria to be sought if the proposed action is denied but dredging is essential and no feasible alternatives are available. EPA has to determine that the proposed dumping will have no unacceptable adverse effect on municipal water supplies, shellfish beds, fishery areas, wildlife areas, or recreational areas before granting the waiver.

2.2 PART 227, SUBPART A: GENERAL

Subpart A defines the applicability of Part 227, Criteria for the Evaluation of Permit Applications for Ocean Dumping of Materials, and establishes general criteria applicable to the disposal of dredged material.

2.3 PART 227, SUBPART B: ENVIRONMENTAL IMPACT

Subpart B sets general and specific criteria that have to be satisfied for disposal of dredged material in the ocean. Subpart B details procedures to be used in evaluating whether dredged material proposed for ocean dumping complies with the applicable provisions of Part 227. Section 227.5 establishes important prohibitions applicable to dredged material.

2.3.1 Trace Contaminants

Section 227.6 prohibits dumping of certain constituents as other than trace contaminants unless they are rapidly rendered harmless. This is a key section of the regulations. TRACE CONTAMINANTS ARE NOT DEFINED IN TERMS OF NUMERICAL CHEMICAL LIMITS, BUT RATHER IN TERMS OF PERSISTENCE, TOXICITY, AND BIOACCUMULATION THAT WILL NOT CAUSE AN UNACCEPTABLE ADVERSE IMPACT AFTER DUMPING. This is expressed in regulatory language in paragraphs 227.6(b) and (c).

By this definition of trace contaminants, marine organisms are regarded, in a sense, as analytical instruments for determining the environmentally adverse consequences (if any) of any contaminants present. This definition of trace contaminants requires that the lack of unacceptable adverse effect in biological studies be taken to mean that contaminants are absent, or present only in amounts and/or forms that are not environmentally active, and therefore do not exceed the trace contaminant definition. When effects occur in dredged-material tests, it is not possible within the present state of knowledge to determine which constituent(s) caused the observed effects. Therefore, it has to be assumed that they are caused by materials described in . 227.6, because it cannot be established that this is not the case. This would mean that one or more contaminants are present in greater than trace concentrations. In practice, the exact identity of the contaminant(s) causing the effect is of little concern under 40 CFR 227 because there should be no ocean disposal of dredged material that causes an unacceptable effect. Following this reasoning, unacceptable bioaccumulation of any potentially harmful constituent, whether listed in . 227.6 or not, could make the dredged material potentially undesirable.

Because assessment of trace contaminants depends upon the determination of the potential for effects, an assessment cannot be made until the impact evaluation is completed and interpreted. Only then can effects, and thus the presence of materials as other than trace contaminants, be determined.

2.3.2 Biological Evaluations

As specified in paragraph 227.13(c), the evaluation process emphasizes potential biological effects, rather than chemical presence, of the possible contaminants. Although bioassays are not precise predictors of environmental effects, they are regarded as the best methods available for integrating the effects of multiple contaminants. Bioassays for

whole sediment evaluation use appropriate sensitive test organisms and record mortality as the endpoint.

Mortality of a certain percent of the organisms of a particular species in a laboratory test does not imply that the population of that species around the disposal site would decline by the same percent if the proposed disposal takes place. However, dredged-material and reference- sediment bioassay results can be compared to determine if the dredged material has significantly higher toxicity. This manual provides guidance under the regulations on determining the magnitude of mortality that may be considered to be a real increase.

Bioaccumulation is included in the required evaluations by paragraphs 227.6(b) and (c) of the regulations. Bioaccumulation indicates biological availability of contaminants in the dredged material. It also assesses the potential for long-term accumulation of contaminants in aquatic food webs to levels that might be harmful to consumers, which could include man, without killing the intermediate organisms. To use bioaccumulation in a decision, it is necessary to predict whether there will be a cause-and-effect relationship between the animal's presence in dredged material and a meaningful adverse elevation of body burden of contaminants above that of similar animals not exposed to the dredged material.

It is difficult to quantify either the ecological consequences of a given tissue concentration of a bioaccumulated contaminant or the consequences of that body burden to the animal. This manual does not provide quantitative guidance on interpreting the ecological meaning of the bioaccumulation observed. Instead, measured bioaccumulation is considered to be potentially unacceptable if animals exposed to the dredged material bioaccumulate statistically greater amounts of contaminants than do animals exposed to reference sediments. Because a statistically significant difference is not a quantitative prediction that an ecologically important impact would occur in the field, this manual presents in Sections 6.3 and 7.2 additional factors to be weighed in evaluating the potential ecological impact of bioaccumulation. This is more likely to result in environmentally sound evaluations than is reliance on statistical significance alone. However, the tests described in this manual can indicate the *potential* for such an ecological impact on a case-specific basis. As pointed out in the preceding discussion of Part 227, Subpart B, the trace-contaminants determination cannot be made until bioaccumulation potential is evaluated.

Biological evaluations serve to integrate the chemical and biological interactions of the suite of contaminants present in a dredged-material sample by measuring their effects on test organisms. In this way, biological methods are more direct and specific than are chemical evaluations, which have to infer interactions and effects based on sediment-contaminant data alone. Within the constraints of experimental conditions and the endpoint of effect measured, biological evaluations provide a quantitative comparison of the effect of a dredged material and acceptable conditions as represented by reference sediments. Thus, a statistically significant result in this comparison indicates that the dredged material in question causes a direct and specific biological effect under test

conditions and, therefore, has the potential to cause an ecologically unacceptable impact. These results will be used to determine the acceptability of the material for ocean disposal.

2.4 PART 227, SUBPART C: NEED FOR OCEAN DUMPING

Subpart C is primarily an evaluation of the need for ocean dumping. Initially, no disposal alternative is considered more desirable than any other, and the evaluation is made on a case-by-case basis. That is, confined or upland disposal cannot be considered environmentally preferable to ocean disposal unless consideration of potential environmental impact (e.g., groundwater contamination, leachate and runoff impact, permanent alteration of the site) shows it to be so. Similarly, ocean disposal cannot automatically be considered the most desirable alternative.

2.5 PART 227, SUBPART D: IMPACT OF THE PROPOSED DUMPING ON AESTHETIC, RECREATIONAL, AND ECONOMIC VALUES

Before a proposed disposal action may be approved, the probable impact on esthetics, recreation, and economic values has to be evaluated, as described in Subpart D, and information from the technical assessment described in Subpart B may be useful. Section 227.19 requires that the results of the Subpart D assessment be expressed, insofar as possible, in quantitative terms.

2.6 PART 227, SUBPART E: IMPACT OF THE PROPOSED DUMPING ON OTHER USES OF THE OCEAN

Subpart E is related to Subpart D, but it requires evaluation of specific actual or potential uses of the disposal-site environs, including but not limited to those listed in . 227.21. These are evaluations for which specific quantitative tests cannot be given. However, much information developed in the Subpart B technical evaluations will be relevant to the assessment of potential impact on living resources and their utilization.

2.7 PART 227, SUBPART G: DEFINITIONS

Subpart G provides definitions for the concepts used in test protocols for performing the evaluations required by .. 227.6 and 227.13 of the regulations. These evaluations are required to determine compliance with the limiting permissible concentration as defined in . 227.27.

2.7.1 Limiting Permissible Concentration

2.7.1.1 Water Column

The limiting permissible concentration (LPC) applicable to potential water-column impact is defined in paragraph 227.27(a). The LPC for the portion of dredged material that will remain in the water column is the concentration of any dissolved dredged-material constituent that, after making allowance for initial mixing, will not exceed applicable marine water-quality criteria (WQC). If WQC have not been established for all of the contaminants of concern in the dredged material, or if synergistic effects are suspected, the LPC is 0.01 of the acutely toxic concentration of dredged material in the water column after the 4-h initial-mixing period [paragraph 227.29(a)]. Chemical analyses are performed for contaminants that may be released from dredged material in dissolved form, and the results are compared against the WQC for these contaminants after making allowance for initial mixing. This provides an indirect evaluation of the potential biological impact because the WQC were derived from toxicity tests of solutions of the various contaminants. In this manual, Section 4.2 discusses identification of contaminants of concern in the water column; Section 8 discusses sample-collection and preservation methods; and Section 9 discusses analytical procedures.

When dredged material contains contaminants of concern for which there are no applicable marine WQC or when synergistic effects are suspected, the material remaining in the water column has to be shown to be nontoxic and nonbioaccumulative after initial mixing. Bioassays provide information on the toxicity of contaminants not included in the WQC, and also indicate possible interactive effects of multiple contaminants. Guidance on conducting water-column bioassays is provided in Section 11 of this manual. Because concern about bioaccumulation focusses on the possibility of impact associated with gradual uptake over long exposure times, primary attention is given to dredged material deposited on the bottom. Bioaccumulation from the material remaining in the water column is generally of minor concern owing to the short exposure time and low exposure concentrations resulting from rapid dispersion and dilution. The discussion of biological evaluations in Section 2.3.2 of this manual is critical to realistically assess the potential for adverse impact on the water column.

2.7.1.2 Benthic Environment

Research conducted by EPA and the USACE since the inception of the MPRSA has shown that the greatest potential for environmental impact from dredged material is in the benthic environment. This is because deposited dredged material is not mixed and dispersed as rapidly or as greatly as the portion of the material that may remain in the water column, and bottom-dwelling animals live and feed in and on deposited material for extended periods. Therefore, the major evaluative efforts should be placed on deposited material and the benthic environment, unless there is reason to do otherwise. This manual uses a conservative approach and uses whole-sediment bioassays to evaluate

potential impact of the solid phase of the dredged material. Chemical analyses of dredged material are needed to determine the presence and concentration of contaminants that might be of environmental concern, including concerns about bioaccumulation. However, at present, chemical analysis cannot be used to directly evaluate the biological effects of any contaminants, or combination of contaminants, present in dredged material because the potential effects of such contaminants depend on their bioavailability. Therefore, animals are used in bioassays to determine the biological availability of and potential for impact of contaminants associated with dredged material. Guidance on conducting bioassays with deposited dredged material is given in Section 11, and bioaccumulation guidance is given in Section 12. Understanding the discussion of biological evaluations in Section 2.3.2 is critical to the realistic assessment of the potential for impact on the benthic environment.

While sediment chemistry cannot be used to predict biological effects, it can be used to identify contaminants of concern. Chemistry can also be used to demonstrate that there is "reasonable assurance that such material has not been contaminated by such pollution [227.13(b)(3)(ii)]."

2.7.2 Estimation of Initial Mixing

Section 227.29 of the regulations describes methods for estimating initial mixing. These methods are applied in evaluating the potential for impact of the portion of dredged material that remains in the water column; all water-quality, water-column bioassay, and bioaccumulation data have to be interpreted in light of initial mixing according to . 227.29. This is necessary since biological effects (which are the basis for water-quality criteria) are a function of the biologically available contaminant concentration and exposure time of the organisms. Laboratory bioassays expose organisms to constant concentrations for fixed periods, whereas in the field both concentration and exposure time to a particular concentration change continuously because of mixing and dilution. Both factors interact to control the degree of biological impact; thus, it is necessary to incorporate the mixing expected at the disposal site into the interpretation of data.

2.7.3 Species Selection

Paragraphs 227.27(c) and (d) specify that water-column bioassays will use appropriate sensitive water-column marine organisms, and benthic bioassays will use appropriate sensitive benthic marine organisms.

Paragraph 227.27(c) defines appropriate sensitive water-column marine organisms as at least one species each representative of phytoplankton or zooplankton, crustacean or mollusc, and fish species chosen from among the most sensitive species accepted by EPA/USACE as being reliable test organisms to determine potential water-column impact. Phytoplankton tests can theoretically indicate the potential for stimulation or inhibition by the dredged material in question. However, phytoplankton tests with the portion of dredged material remaining in the water column are extremely difficult to conduct and interpret. This is caused by interferences and predation on the test species by

protozoa in the dredged material being tested. It is widely believed that potential effects on phytoplankton are generally of little environmental concern at ocean dredged-material disposal sites, because of to the extremely variable characteristics of natural phytoplankton assemblages and to the rapid mixing and dilution that occurs in the water column. Therefore, unless there is a specific reason to be concerned about the potential effects of the proposed operation on phytoplankton, this manual recommends that a zooplankton species be selected to fulfill that portion of the species requirement. Laboratory procedures for conducting water-column bioassays are given in Section 11.

Paragraph 227.27(d) defines appropriate sensitive benthic marine organisms as at least one species each representing filter-feeding, deposit-feeding, and burrowing species chosen from among the most sensitive species accepted by EPA/USACE as being reliable organisms to determine potential benthic impact. These are broad, overlapping categories, and this manual recommends different species for bioassays and bioaccumulation testing. Whole-sediment bioassay species generally should include a deposit-feeding amphipod and a polychaete. Bioaccumulation tests generally should include a deposit-feeding bivalve mollusc and a burrowing polychaete. Procedures for conducting bioassays are given in Section 11, and bioaccumulation procedures are given in Section 12.

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Part II. Evaluation of Potential Environmental Impact

3.0 OVERVIEW OF TESTING AND EVALUATION

3.1 REFERENCE AND CONTROL SEDIMENTS

It is important to distinguish clearly between reference and control sediments in the context of testing for benthic impact. Test procedures are conducted on the control and reference sediments in the same way as on the dredged material proposed for ocean disposal.

3.1.1 Control Sediments

Control sediment is a natural sediment essentially free of contaminants. The essential characteristic of control sediment is that it be fully compatible with the needs of the test organisms such that it have no discernible influence on the response being measured in the test. The results of the control-sediment tests are used to verify the health of organisms used in testing and the acceptability of test conditions. Excessive mortality in the control sediment indicates a problem with testing conditions or organisms and can invalidate the corresponding test results.

3.1.2 Reference Sediment

Reference sediment is the key to evaluating the benthic effects of dredged material. Results of tests using reference sediment provide the point of comparison (reference point) against which effects of dredged material are compared. A determination of the potential for dredged material proposed for disposal to cause unacceptable adverse impact can be made by comparing results of tests using reference material to the results of tests using dredged material.

A reference sediment is a sediment, substantially free of contaminants, that is as similar to the grain size of the dredged material and the sediment at the disposal site as practical, and reflects conditions that would exist in the vicinity of the disposal site had no dredged-material disposal ever occurred, but had all other influences on sediment condition taken place. For optimal evaluation of the toxicity and bioaccumulation potential of a dredged material, these reference-sediment conditions have to be met to the maximum extent possible. If it is not possible to fully meet these conditions, tests should use organisms that are not sensitive to grain-size differences among the reference sediment, control sediment, and dredged material. The reference sediment serves as a

point of comparison to identify potential effects of contaminants in the dredged material. It may be appropriate to test more than one reference sediment to evaluate a single dredging project.

3.1.2.1 Reference-Sediment Sampling Location

According to the definition in Section 1.6, reference sediment is substantially free of contaminants, as similar as practical to the grain size of the dredged material and the sediment at the disposal site, and reflects conditions that would exist in the vicinity of the disposal site had no dredged-material disposal ever taken place, but had all other influences on sediment condition occurred. With this in mind, reference sediment is collected outside the boundaries of the dredged-material disposal site, but near enough to the disposal site that the reference sediment is in the same water mass and subject to all the same influences (except previously disposed dredged material) as the disposal site. If there is a potential for sediment migration, reference sediment should not be collected from the area outside the disposal site in the direction of net sediment transport.

Reference sediment may be collected from a single reference-sediment sampling point that satisfies the conditions in this section and meets the requirements of the reference-sediment definition in Section 1.6. This is known as the reference-point approach.

Alternatively, reference sediment may be collected from a number of locations within a reference area that satisfies the conditions in this section and meets the requirements of the reference-sediment definition in Section 1.6. This is known as the reference-area approach.

In the reference-area approach, the reference location is viewed not as a single station or point but as the entire area in the environs of the disposal site, excluding the disposal site itself. Rather than characterize the reference area by sampling at a single point, it is characterized by a number of samples taken throughout the reference area. The intensity of the reference-sediment sample gathering should be tailored to the physical, chemical, and biological characteristics of the disposal site, particularly the dispersal characteristics of the site. Reference-area samples may be composited according to the compositing guidance in Section 8.2.4. The composited or individual samples are then tested for chemistry, toxicity, and bioaccumulation by the same methods used for dredged-material testing. The reference data thus generated are compared to the corresponding dredged-material data in the same way that reference data have traditionally been used.

3.1.2.2 Reference-Sediment Sampling Interval

Reference sediment has to be collected and tested at the time of each dredged-material test if the reference-point approach is used. In this approach, a new sample of reference sediment is collected from the specified reference-sediment sampling point for each test or test series and is tested simultaneously with the dredged material being evaluated.

Logistical considerations might make it impractical to use the reference-area approach at

the time of each test. Reference-area sampling may be conducted periodically as part of a monitoring/management plan for a disposal site. Reference sediment is collected from the reference area, and all appropriate chemistry, bioassay, and bioaccumulation tests are performed on it. The reference data thus generated are used as the basis for evaluating all dredged material tested during some specified period. The reference area is resampled and retested to update the reference data as appropriate.

Using the periodic reference-area approach, reference data are established for each disposal site and for each type of test. To conduct the evaluations put forth in this manual, reference-area data for the proposed dredged material for the specified disposal period must be established for

- Test-species benthic toxicity
- Test-species benthic bioaccumulation period
- Each contaminant that is likely to be of concern at that site.

Development of reference data using all appropriate species and contaminants for all dredged material that may be proposed for a disposal site during the specified period will require planning and coordination. However, most ocean-disposal sites receive dredged material from relatively few locations so that standardization of species for testing and advance identification of potential contaminants of concern for bioaccumulation should be possible.

3.1.2.3 Reference-Sediment Sampling

The importance of thoughtful selection of the approach to reference-sediment sampling cannot be overemphasized. To ensure that the reference sediment is properly located, information gathered during the site-designation process or other similar studies should be completed for both the disposal site and the reference area. Information on the potential for migration of dredged material from the disposal site is particularly important in this regard.

A well-designed sampling plan is essential to the collection, preservation, and storage of samples so that potential toxicity and bioaccumulation can be accurately assessed (see Section 8). The implementation of such a plan is equally essential for dredged material, control sediment, and reference-sediment sampling. The sample collection, preservation, and storage guidance of Section 8 is applicable to dredged material, control sediment, and reference sediment.

3.2 TIERED TESTING AND EVALUATION

The tiered approach to testing used in this manual is designed to aid in generating necessary toxicity and bioaccumulation information, but not more information than is necessary. This allows optimal use of resources by focusing the least effort on dredging operations where the potential (or lack thereof) for unacceptable adverse impact is clear,

and expending the most effort on operations requiring more extensive investigation to determine the potential (or lack thereof) for impact. To achieve this objective, the procedures in this manual are arranged in a series of tiers, or levels of intensity of investigation. The initial tier uses readily available information that may be sufficient for evaluation in some cases. Dredging operations that obviously have low environmental impact generally should not require intensive investigation to reach a decision. Evaluation at successive tiers is based on more extensive and specific information that may be more time-consuming and expensive to generate, but that allows more and more comprehensive evaluations of the potential for environmental effects.

A tiered, or hierarchical, approach to testing and evaluation allows the use of a necessary and sufficient level of testing for each specific dredging operation. The initial tiers (Tiers I and II) use existing information and relatively simple, rapid procedures for determining potential environmental impact of the dredged material in question. For certain dredged materials with readily apparent potential for environmental impact (or lack thereof), information collected in the initial tiers may be sufficient for making decisions. However, more extensive evaluation (Tiers III and IV) may be needed for other materials with less clear potential for impact or for which the information is inadequate. Successive tiers incorporate more intensive evaluation procedures that provide more detailed information about potential impact of the dredged material. The intent of the tiered approach is to use resources efficiently by testing only as intensely as is necessary to provide sufficient information for making decisions. The tiered approach minimizes excessive testing of dredging operations for which this is unnecessary and appropriately directs more intense testing to operations that require more technical information for evaluation. Tiered testing results in more efficient completion of required evaluations and reduced costs, especially to low-risk operations.

It is neither necessary nor desirable that all dredged material be evaluated through all tiers in sequence. If information warrants, it is acceptable to proceed directly to Tier II, III, or IV. It is also fully acceptable to carry water-column and benthic evaluations, or toxicity and bioaccumulation evaluations, to different tiers to generate the information necessary and sufficient to determine compliance with the regulations.

Prior to initiating testing, it is essential that the informational requirements of preceding tiers be thoroughly understood and that the information necessary for decision-making at the advanced tier be assembled. For example, it is always appropriate to gather all relevant available information and identify the chemicals of concern for the dredged material in question. Although these activities are components of Tier I, they have to be conducted even if a complete evaluation at the initial tiers is not considered appropriate. Similarly, water-column evaluations require that Tier II be completed to obtain information sufficient for an LPC determination in Tier II, III, or IV.

It is necessary to proceed through the tiers only until information sufficient to determine compliance or noncompliance with .. 227.6 and 226.13 has been obtained. For example, if the available information is sufficient to demonstrate that the LPC is met, no further testing is required. Similarly, if historical data have consistently shown a particular

dredged material to exceed the LPC, an exhaustive evaluation may not be warranted. After any of the first three tiers is completed, one of three decisions can be made according to the evaluative guidance in Sections 4 through 7 of this manual: (1) information is sufficient to determine that the LPC is met, (2) information is sufficient to determine that the LPC is not met, or (3) information is insufficient to make a determination. In the last case, if ocean disposal is still to be considered, the evaluation would proceed to a higher tier for further testing. In unusual circumstances, where a compliance determination cannot be made after completion of the first three tiers, further testing in Tier IV may be appropriate. Tier IV tests have to be carefully designed to supply all information necessary to make a determination on whether the dredged material meets the LPC.

If the information is insufficient to determine LPC compliance after completing Tier I, II, or III, further testing is not required if noncompliance with the LPC is assumed.

The Tier I evaluation helps to identify the needed information and to determine appropriate tiers and tests necessary to collect this information. In all cases, it is appropriate to gather the information used in Tier I, although it may be clear without formal Tier I evaluation that further assessment will be necessary. It is, however, always necessary to identify the contaminants of concern, if any, at the Tier I level. Tiers I, II, and III are intended to suffice for almost all evaluations. Tier IV is intended only for extremely rare occasions.

With some dredged materials, biological effects will be easily determined, but bioaccumulation potential will require more investigation, or vice versa. In other cases, determining potential benthic effects may require more investigation than evaluating water-column effects. The tiered-testing approach used in the manual accommodates such situations by providing independent evaluation of biological effects and bioaccumulation and of water-column and benthic effects only to the extent needed to make a decision about each.

The tests in the tiers presented in the manual reflect the present state-of-the-art evaluation procedures for dredged-material evaluation. The procedures will be improved and updated as scientific knowledge increases. Part III of this manual provides the testing guidance for each tier, and includes specific guidance on topics such as test selection, test design and conditions, determining acceptability of tests, and statistical frameworks for interpretation of results. Here, in Part II, evaluative guidance is provided for using bioassay and bioaccumulation data from each tier of testing to determine compliance with the regulations.

It is important to emphasize that testing at every tier is not required for every situation. However, evaluations conducted in Tiers II, III, and IV may utilize information that was collected in preceding tiers. Thus, skipping tiers may not produce any time or resource savings. At any tier, failure to satisfactorily determine the potential for unacceptable environmental impact results in additional testing at a subsequent, more complex tier unless a decision is made to seek other disposal alternatives. If there is reason to believe that there is contamination and that the available information is not adequate to support a

decision, testing can begin at Tier II, III, or IV without conducting the evaluation at each preceding tier. It would be extremely unusual to go directly to Tier IV. The tiered-testing approach permits the flexibility to evaluate dredged materials in the most efficient way. More complex evaluation techniques are necessary only in those situations where the potential effects of contaminants in the dredged materials can be evaluated only with additional technical information.

Although the tiered-testing approach outlined in this manual provides an effective means of implementing the regulations, it is recognized that the evaluation of dredged material is an evolving field. It is anticipated that, as new methods of evaluation are developed and accepted, they can be integrated into the tiered framework. With the advent of acceptable new evaluation procedures, the tiered approach will be maintained because of the efficiency afforded by its hierarchical design.

The tiered approach used in the manual is summarized in Figure 3-1, and additional detail on water-column and benthic evaluation is presented in Figures 3-2 and 3-3. These flowcharts should be used in conjunction with a careful reading of the corresponding guidance presented in the text. The Sections in the manual that present the technical and decision-making guidance shown by the flowcharts are indicated in the boxes on the Figures.

The following discussion briefly overviews the testing and evaluation guidance in the manual, and integrates the Figures with the text. By necessity, this overview is not detailed, and cannot be used on a standalone basis for regulation.

As illustrated in Figure 3-1, the evaluation begins in Tier I with the compilation of all available information relevant to the operation in question (Section 4.1). If the chemical information is not adequate, a chemical analysis of the dredged material should be performed on contaminants of concern. Information collected in Tier I is evaluated to determine whether it is sufficient for decision-making, as described in Section 4.3. If the information is sufficient, a determination is made (Figure 3-1) as to whether the material is (1) sand, (2) suitable for beach nourishment, or (3) similar to the disposal site and from an area far removed from pollution sources (Section 4.3). If so, the material meets the paragraph 227.13(b) criteria, meets the LPC, and is acceptable for ocean disposal at a designated site if all other requirements of the regulations are satisfied. If not, the existing information (which has already been judged sufficient for decision-making) is used to determine whether the dredged material can be disposed without exceeding the LPC in compliance with paragraph 227.13(c) of the regulations (Figure 3-1 and Section 4.3). This is the same standard used to judge acceptability in Tiers II-IV when new data are necessary.

If, in Tier 1, the dredged material is found to meet the LPC and paragraph 227.13(c), no further information on contaminants is required to determine compliance. Alternatively, the dredged material may be found to not meet the LPC and paragraph 227.13(c). In either case, the decisions on whether such material might be allowable for ocean disposal under the MPRSA and other applicable regulations, and the procedural steps to make this

determination, are issues beyond the scope of this manual. If the initial information is insufficient for determining compliance, further evaluation in Tiers II, III, and/or IV, as necessary, is required (Figures 3-2

and 3-3).

If water-column impact cannot be fully evaluated in Tier I, completion of Tier II is mandatory to determine compliance with applicable marine water-quality criteria (WQC) (Figure 3-2). This evaluation is conducted by entering the known contaminant concentrations into a numerical mixing model as described in Section 10.1.1. The sediment-concentration data entered in the model at this point are those which were identified in the Tier I evaluation. Total release of the contaminants into the water column is assumed, thereby using the model as a screen and being able to show LPC compliance for dredged material that will cause very little impact on the water column. However, if the model screen predicts that the WQC will be exceeded, an elutriate test must be conducted and the results from the sediment chemical analysis and evaluation used to determine the concentration of contaminants that might enter the water column during a disposal operation (Section 10.1.2). Following the sediment chemical analysis, the model is run a second time, using the elutriate chemical data that more closely represent the available contaminants. If the model predicts again that the WQC will be exceeded, the LPC for WQC compliance is not met. Conversely, if the model shows that the WQC are not exceeded, the LPC is met for WQC compliance. However, when there are no WQC for all contaminants of concern, or synergistic effects are suspected among the contaminants, water-column impact must also be evaluated by toxicity testing [paragraph 227.13(c)(2)(ii)] in Tier III.

In Tier II, the potential for benthic impact related to bioaccumulation of nonpolar organic compounds is evaluated according to the guidance in Section 10.1 (Figure 3-3). This involves calculation of theoretical bioaccumulation potential (TBP) of nonpolar organic compounds based on partitioning between the organic carbon in sediments and the lipids in organisms (see Section 10.2). If the TBP is lower from the dredged material than from the reference sediment, further testing for bioaccumulation of these nonpolar organic contaminants is not required. If the TBP of the dredged material exceeds that of the reference sediment, or if there are contaminants of concern that are not nonpolar organics, bioaccumulation testing in Tiers III and/or IV is required (Section 5.2 and Figure 3-3).

It should be recognized that Tier II consists only of a numerical model to determine compliance with the WQC and a calculation to estimate the TBP for nonpolar organic compounds. As presently structured, Tier II cannot be used to fully determine LPC compliance for dredged material. Research is being conducted to develop new water-column and benthic tests for this tier which will allow more definitive LPC evaluations.

Tier III water-column testing consists of evaluation of the toxicity of the suspended and dissolved portions of the dredged material that remain in the water column, after consideration of initial mixing (see Section 11.1 and Figure 3-2). If the model predicts

that the dredged-material concentration remaining in the water column after initial mixing is greater than 0.01 of the corresponding LC50, the LPC for water-column impact is not met (see Section 6.1 and Figure

3-2). If the predicted concentration is less than 0.01 of the LC50, the LPC for water-column impact is met and compliance is further assessed for benthic impact and other regulations (see Section 6.1 and Figure 3-2).

Tier III benthic tests consist of acute toxicity bioassays (Section 11.2) and bioaccumulation tests (Section 12.2), as illustrated in Figure 3-3. When sublethal chronic tests are approved for dredged-material evaluation, they will be incorporated into this Tier. At present, benthic impact is evaluated by comparing dredged-material toxicity against the reference sediment (Figure 3-3). The LPC is not met for benthic toxicity (Section 6.2, and Figure 3-3) if the dredged-material toxicity (1) is statistically greater than the reference sediment and (2) exceeds reference-sediment toxicity by at least 10%-20% (see Section 6.2 for the applicable percentage). This approach is discussed in more detail in Section 6.2. The LPC for benthic toxicity is met if the toxicity of the dredged material does not statistically exceed that of the reference material by more than the applicable percentage (Section 6.2 and Figure 3-3).

Bioaccumulation of dredged-material contaminants of concern is assessed in Tier III by comparing the bioavailability of the contaminants against the Food and Drug Administration Action Levels for Poisonous and Deleterious Substances in Fish and Shellfish for Human Food and to the bioavailability of contaminants in the reference sediment. If any of the FDA levels is statistically exceeded (Section 6.3 and Figure 3-3), the LPC is not met for bioaccumulation. If results show that the FDA levels are not exceeded but that the reference-sediment values are exceeded, further evaluation using case-specific criteria is required (Section 6.3 and Figure 3-3). The case-specific criteria are to reflect the local information that addresses the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) of the regulations. If results show that neither the FDA levels nor reference-sediment values are exceeded, the dredged material meets the LPC for bioaccumulation. The purpose of this case-specific evaluation in Tier III is to reach an environmentally sound LPC evaluation for bioaccumulation without having to commit additional time and resources under Tier IV testing, unless necessary.

Tier IV bioassay testing is intended only for infrequent application, under unusual circumstances that warrant specifically designed case studies (Figure 3-1). Tier IV water-column and benthic bioassays are discussed in Section 11, and interpretive guidance is discussed in Sections 7.1 and 7.2. Tier IV benthic and water-column bioassays have to be interpreted in relation to case-specific criteria (Figures 3-2 and 3-3) developed as discussed in Section 7.1. Tier IV bioaccumulation evaluation consists of determination of steady-state bioaccumulation of dredged-material contaminants (Figure 3-3), as described in Section 12.2. If a steady-state body burden statistically exceeds an FDA level for a single contaminant, the LPC for bioaccumulation is not met (Section 7.2 and Figure 3-3). If the body burdens of animals exposed to the dredged material do not exceed any FDA levels or the body burdens of the reference animals, the LPC is met

(Section 7.2 and Figure 3-3). Animal body burdens not statistically exceeding FDA levels but statistically higher than those of the reference-sediment animals are compared to the body burdens in similar organisms living around, but not in, the proposed disposal site. If the body burdens from the dredged-material animals do not statistically exceed the body burdens of these field organisms, the LPC is met (Section 7.2 and Figure 3-3). If body burdens from the dredged-material animals exceed those of field organisms, case-specific criteria for the dredging operation must be developed (Section 7.2 and Figure 3-3). Evaluation of body burdens using the case-specific criteria in Tier IV provides for a yes/no compliance evaluation with the LPC for bioaccumulation.

If the above procedures show that the LPC cannot be met, management-action alternatives will have to be considered if the ocean-disposal option is to be pursued. Management actions are project-specific and are addressed in other EPA/USACE documents. The decisions as to whether such material might be allowable for ocean disposal under the MPRSA and other applicable regulations, and the procedural steps to be followed in making this determination, are issues that are beyond the scope of this manual.

In summary, the tiered, or hierarchical, testing approach presented in this manual allows the appropriate level of testing to be used for each specific dredging operation.

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4.0 TIER I

The purpose of Tier I is to determine whether a decision on compliance with the limiting permissible concentration (LPC) can be made on the basis of existing information. Tier I is a comprehensive analysis of all existing and readily available, assembled, and interpreted information on the proposed dredging project, including all previously collected physical, chemical, and biological data. Part III of this manual, particularly Sections 9, 10, 11, and 12, is to be consulted when evaluating the information obtained during Tier I evaluations.

If the information set compiled in Tier I is complete and comparable to that which would appropriately satisfy Tier II, III, or IV, a decision on LPC compliance can be completed without proceeding into the higher tiers (Figure 3-1). For an LPC evaluation to be completed within Tier I, the weight of evidence of the collected information must convincingly show that the dredged-material disposal will or will not meet the LPC.

For a Tier I evaluation, the information collected on the proposed dredged material is first compared to the three exclusionary criteria in paragraph 227.13(b). If one or more of the exclusionary criteria can be satisfied, the LPC is met for the dredged material and no further evaluation is required. If no exclusionary criteria can be met, the LPC is evaluated based on the collected information. This information must include data analyses of the toxicity and bioaccumulation potential of the dredged material and of the reference sediments. The information must also be sufficient to determine if the WQC or 1% of the LC50 will be exceeded in the water-column following the initial-mixing period. If there is not adequate information available for a Tier I LPC evaluation, the evaluation process moves to Tier II.

It is important to note that, even if a final LPC evaluation is not reached within Tier I, the information collected can be put to use in later tier analyses. A primary purpose of Tier I is to identify the contaminants of concern (if any) in that particular dredged material. This information is used to select analyses in Tiers II, III, and IV. Similarly, other information collected in Tier I may be used to satisfy all or portions of evaluations in other tiers. It is necessary to proceed through the tier-testing mechanism only until a definitive LPC evaluation is reached for potential water-column impact and for the toxicity and bioaccumulation components of benthic impact. Rigorous information collection and assessment in Tier I inevitably saves time and resources in making final LPC determinations.

Annual or episodic dredging, undertaken to maintain existing navigation improvements, may warrant a Tier I reevaluation prior to each episode. The recommendation of EPA and the USACE is that the interval between reevaluation of Tier I data for these projects not exceed

3 years. This reevaluation minimally should include reassessment of all new and previously evaluated physical and chemical data relative to any regulatory changes, changes in sediment composition or deposition (e.g., industrial development in the watershed), improvements in analytical methods and contaminant detectability, and quality-assurance considerations.

4.1 COMPILATION OF EXISTING INFORMATION

The focus of the Tier I evaluation is on paragraph 227.13(b) and the potential for contaminant-associated impact upon ocean dumping. The information-gathering phase of Tier I evaluations has to be as complete as is reasonably possible, and existing information from all reasonably available sources has to be included. Although there are no minimum requirements, a more complete inventory of available information will increase the likelihood that decisions concerning the impact of dredged material may be made at initial tiers. Sources of available information include the following, without limitation.

- Available results of prior physical, chemical, and biological tests of the material proposed to be dumped.
- Available results of prior field monitoring studies of the material proposed to be dumped (e.g., physical characteristics, organic-carbon content, and grain size).
- Available information describing the source of the material to be dumped which would be relevant to the identification of potential contaminants of concern.
- Existing data contained in files of either the EPA or USACE or are otherwise available from public or private sources. Examples of sources from which relevant information might be obtained include
    - Selected Chemical Spill Listing (EPA)
    - Pesticide Spill Reporting System (EPA)
    - Pollution Incident Reporting System (United States Coast Guard)
    - Identification of In-Place Pollutants and Priorities for Removal (EPA)
    - Hazardous waste sites and management facilities reports (EPA)
    - USACE studies of sediment pollution and sediments
    - Federal STORET, BIOS, CETIS, and ODES databases (EPA)
    - Water and sediment data on major tributaries (Geological Survey)
    - NPDES permit records
    - CWA 404(b)(1) evaluations
    - Pertinent and applicable research reports
    - MPRSA 103 evaluations
    - Port Authorities
    - Colleges/Universities

- Records of State environmental agencies
- Published scientific literature

Evaluation of all reasonably available information allows determination of the potential for contaminants to have been introduced to the dredged material. This information, evaluated with consideration of the physical nature of the dredging site, dredged material, and the proposed disposal site, allows a determination of whether the dredged material complies with paragraph 227.13(b) (Appendix A). Decisions about compliance will be made on a case-by-case basis for each proposed disposal operation, and specific quantitative guidance applicable to all situations nationwide cannot be offered. More detailed guidance for reaching decisions about compliance may be developed by the EPA Region and USACE District by considering available scientific information and locally important concerns. This information will be important in reaching an administrative decision that complies with the requirements of paragraph 227.13(b). In evaluating the likelihood that disposal of a dredged material may cause contaminant-associated impact, concern decreases with the increase of factors such as

- Isolation of the dredging operation from known existing and historical sources of pollution
- Time since historical sources of pollution have been remediated
- Number and frequency of maintenance dredging operations since abatement of the source of contamination
- Mixing and dilution occurring between the contamination source and the dredging site
- Transport and potential deposition of sediment in the dredging area from sources other than those potentially affected by contamination
- Grain size of the dredged material.

Concern regarding contaminant-associated impact increases with the increase of factors such as the number, amount, and toxicological importance of contaminants

- Known to have been introduced to the dredging site
- Suspected to have been introduced to the dredging site
- With continuing input from existing sources
- From historical sources no longer active.

These and other considerations are complexly interrelated; i.e., the acceptable degree of isolation from sources of pollution depends on the number, amount, and toxicological importance of the contaminants as well as on all other factors. These considerations have to be evaluated for all dredged material. Even so, it is desirable that local guidance be developed, based on technical evaluations, that describes the emphasis on factors deemed appropriate in each area. In all cases, the decisions that are based on these factors have to comply with the requirements of paragraph 227.13(b).

4.2 IDENTIFICATION OF CONTAMINANTS OF CONCERN

In the Tier I decision sequence (Figure 3-1), the first possibility is that more information is required to determine compliance with the regulations. A critical prerequisite to generating this information is deciding, on a case-by-case basis, which contaminants are of concern in the particular dredged material being evaluated. To determine the contaminants of concern, it may be necessary to supplement available information with additional chemical analyses of the dredged material.

On a National scale, dredged material may contain a variety of chemicals. It is difficult to specify a single set of contaminants that adequately addresses all environmental concerns about all dredged materials in the country. The contaminants of concern in a particular dredged material have to be identified on a case-by-case basis. In some dredged materials, there may be no contaminants of concern. Different dredging operations may have their own set of contaminants of environmental concern that should be adequately evaluated for each operation. The selection of the appropriate contaminants of concern for each dredged material is crucial to the success of the testing program.

Identifying specific contaminants, if any, that are of concern in a particular dredged material is dependent on the information collected for Tier I, which provides a preliminary basis for determining potential contamination of the dredged material. In some instances, it may be sufficient to perform confirmatory analyses for specific contaminants of concern. In other cases, where the initial evaluation indicates that a variety of contaminants of concern may be present, chemical analysis of the dredged material could provide a useful inventory, and a bulk-chemical analysis conducted according to the guidance in Section 9.3 may be appropriate and, in fact, would be necessary to conduct Tier II.

From the list of contaminants shown to be potentially present in a dredged material, it is necessary to determine which specific contaminants are of concern in terms of potential environmental impact. Some contaminants are always of interest because of the provisions of the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter (London Dumping Convention; LDC) and the incorporation of these contaminants into the Marine Protection, Research, and Sanctuaries Act of 1972 (MPRSA) and .. 227.5 and 227.6 of the regulations. In identifying contaminants of concern, the contaminants necessary to determine compliance with the requirements of . 227.6 of the regulations have to be included. Other contaminants that should be included are those that might reasonably be expected to cause unacceptable adverse impact if the dredged material in question were placed in the ocean.

Current knowledge is inadequate to predict biological effects based on the presence of contaminants in dredged material. Therefore, those chemicals identified as contaminants of concern are evaluated according to the biological effects-based criteria in . 227.13 (Appendix A). Sediment-chemistry data describing the concentration of contaminants of concern should not be directly used to make decisions regarding the acceptability of dredged material for ocean disposal. This information should be considered when

selecting appropriate bioassay/ bioaccumulation testing procedures and species to be evaluated, and when reviewing the results obtained from these tests. That is, the presence and levels of contaminants of concern can be used on a case-by-case basis when reviewing the validity of bioassay/bioaccumulation results. Chemistry data should be used only as a feedback trigger to indicate the need for further evaluation of quality assurance/quality control (QA/QC) to assist in determining if the bioassay/bioaccumulation tests to determine if the tests were properly conducted. If the QA/QC review indicates that the tests were improperly conducted, retesting would be appropriate.

The contaminants of concern in each dredged material should be identified on the basis of the following, keeping in mind the discussion in Sections 9.3 and 9.4 and the requirements of . 227.6 of the regulations:

- Presence in the dredged material
- Presence in the dredged material relative to the concentration in the reference material
- Toxicological importance
- Persistence in the environment
- Propensity to bioaccumulate from sediments
- The major chemical properties controlling the propensity to bioaccumulate are

Hydrophobicity

Literally, "fear of water"; the property of neutral (i.e., uncharged) organic molecules that causes them to associate with surfaces or organic solvents rather than to be in aqueous solution. The presence of a neutral surface such as an uncharged organic molecule causes water molecules to become structured around the intruding entity. This structuring is energetically unfavorable, and the neutral organic molecule tends to be partitioned to a less energetic phase if one is available. In an operational sense, hydrophobicity is the reverse of aqueous solubility. The octanol/water partition coefficient (Kow, log Kow, or log P) is a measure of hydrophobicity. The tendency for organic chemicals to bioaccumulate is related to their hydrophobicity. Bioaccumulation factors increase with increasing hydrophobicity up to a log Kow of about 6.00. At hydrophobicities greater than about log Kow = 6.00, bioaccumulation factors tend to not increase due, most likely, to reduced bioavailability.

Aqueous Solubility

Chemicals such as acids, bases, and salts that speciate (dissociate) as charged entities tend to be water-soluble and those that do not speciate (neutral and nonpolar organic compounds) tend to be insoluble, or nearly so. Solubility favors rapid uptake of chemicals by organisms, but at the same

time favors rapid elimination, with the result that soluble chemicals generally do not bioaccumulate to a great extent. The soluble free ions of certain heavy metals are exceptional in that they bind with tissues and thus are actively bioaccumulated by organisms.

Stability

For chemicals to bioaccumulate, they must be stable, conservative, and resistant to degradation. Organic compounds with structures that protect them from the catalytic action of enzymes or from nonenzymatic hydrolysis tend to bioaccumulate. Phosphate ester pesticides do not bioaccumulate because they are easily hydrolyzed. Unsubstituted polynuclear aromatic hydrocarbons (PAH) can be broken down by an initial enzymatic opening of ring structures. The presence of electron-withdrawing substituents tends to stabilize an organic molecule. Chlorines, for example, are bulky, highly electronegative atoms that tend to protect the nucleus of an organic molecule against chemical attack. Chlorinated organic compounds bioaccumulate to high levels because they are easily taken up by organisms, and, once in the body, they cannot be readily broken down and eliminated.

Stereochemistry

The spatial configuration, i.e., stereochemistry, of a neutral molecule affects its tendency to bioaccumulate. Molecules that are planar tend to be more lipid- soluble (lipophilic) than do globular molecules of similar molecular weight. For neutral organic molecules, planarity generally correlates with higher bioaccumulation unless the molecule is easily metabolized by an organism.

These and other considerations important to identifying contaminants of concern are complexly interrelated and have to be evaluated individually for each dredged material. Even so, it is desirable that local guidance be developed, based on technical evaluations, that describes the emphasis on various factors deemed appropriate for identifying contaminants of concern in each area. In all cases, the decisions based on these factors have to comply with the requirements of . 227.13 (Appendix A).

4.3 DETERMINATION OF COMPLIANCE

After consideration of all available information, one of the following conclusions is reached (Figure 3-1).

- Existing information does not provide a sufficient basis for making a decision about whether dredged material complies with . 227.13 of the regulations. In this case, further evaluation in Tiers II, III, and/or IV is appropriate.
- Existing information provides a sufficient basis for making a decision about

whether the dredged material complies with . 227.13 of the regulations.

In the latter case, based on consideration of available information, one of the following conclusions is reached (Figure 3-1).

- The material complies with the paragraph 227.13(b) criteria for exclusion from further testing (Appendix A). If so, no further information on contaminants is necessary to determine compliance.
- The material does not comply with the paragraph 227.13(b) criteria, but does comply with the paragraph 227.13(c) criteria and the limiting permissible concentration (Appendix A). If so, no further information on contaminants is necessary to determine compliance.
- The material does not comply with either the paragraph 227.13(b) or the paragraph 227.13(c) criteria and with the LPC (Appendix A). If so, no further information is necessary to determine noncompliance.

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5.0 TIER II EVALUATION

Tier II consists of evaluation of marine water-quality criteria (WQC) compliance using a numerical mixing model of the dump-site conditions (Figure 3-2 and Appendix B) and an evaluation of the potential for benthic impact using calculations of theoretical bioaccumulation potential (Figure 3-3 and Section 10.2). The purpose of Tier II is to provide a reliable, rapid screen for potential impact and thereby eliminate the need for further testing. The dredged-material impact in the water column must be within the applicable marine WQC for all contaminants of concern outside the boundary of the site at all times and within the site following the 4-h initial-mixing period (Figure 3-2). When there are no WQC for all contaminants of concern, or when synergistic effects are suspected between the contaminants, water-column impact must also be investigated by toxicity testing [paragraph 227.13(c)(2)(ii)) in Tier III (Figure 3-2). Current WQC for the protection of marine life can be obtained from the U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Criteria and Standards Division, Standards Branch (WH-585), 401 M Street S.W., Washington, DC 20460.

For benthic evaluations, there is not widespread agreement that any single dredged-material evaluation procedure fully satisfies the objective of and is suitable for use in Tier II. When technically sound sediment quality criteria (SQC) are developed and the corresponding Final Notice of Availability is published in the *Federal Register* by EPA, these criteria will be incorporated into Tier II benthic-impact evaluations. The incorporation of these criteria into Tier II will be implemented by the insertion of a new Section into this testing manual. This new Section will be developed jointly by EPA and the USACE. It will provide guidance on how to use the SQC to determine compliance with the limiting permissible concentration (LPC).

At present, only the bioaccumulation impact of nonpolar organic compounds in dredged material on benthic organisms can be evaluated in Tier II (Figure 3-3). The approved procedure calculates the theoretical bioaccumulation potential (TBP) for a test organism by factoring the concentrations of the nonpolar organic chemical and the total organic carbon (TOC) in the sediment and the percent lipid concentration (%L) in the organism. This calculation predicts the magnitude of bioaccumulation likely to be associated with nonpolar organic contaminants in the dredged material.

5.1 WATER-COLUMN EVALUATIONS

Program experience has shown that in most cases the existing data are sufficient to make water-column LPC determinations. However, Tier I evaluation may show that the existing information is insufficient to evaluate LPC compliance. In this case, paragraph 227.13(c) of the regulations (Appendix A) requires testing to determine the potential for

water-column impact and whether the corresponding LPC is met. This evaluation is performed under Tier II. If a WQC LPC decision cannot be made in Tier I, Tier II evaluation is mandatory even if subsequent evaluations are to be conducted in Tiers III and IV (Figure 3-2). Under no circumstances can the disposal of the dredged material cause the applicable marine WQC to be exceeded outside the disposal site at any time or within the site after the 4-h initial-mixing period. The WQC evaluation in Tier II can be bypassed only if there are no WQC for any of the contaminants in the dredged material.

The Tier II water-column evaluation for WQC is a two-step process, using the numerical model provided in Appendix B. The first step uses the model as a screen and assumes that all of the contaminants in the dredged material are released into the water column during the disposal process. The second step applies the same model with results from chemical analysis of the elutriate test.

5.1.1 Step 1: Screen To Determine WQC Compliance

Step 1 of the Tier II water-column evaluation comprises a screen that assumes that all of the contaminants in the dredged material are released into the water column during the disposal operation (Section 10.1.1). This is a conservative assumption because, in virtually all cases except at extremely deep disposal sites, most of the contaminants remain within the dredged material that settles to the bottom. If the numerical model (Appendix B) predicts that the concentration of all contaminants of concern released into the water are less than the applicable WQC and if no synergistic effects are suspected, the dredged material meets the LPC for the water column. If the screen/model, as applied in Step 1, indicates that the LPC is exceeded, Step 2 is employed, as described in Section 5.1.2. If WQC have not been established for all contaminants of concern or if synergistic effects are suspected, further testing in Tier III is required to determine compliance with the LPC for the water column (Section 6.1).

5.1.2 Step 2: Elutriate Analysis To Determine WQC Compliance

If additional water-column testing of dredged material is determined to be necessary after completion of the screen (Section 5.1.1), the regulations (Appendix A) are very specific about tests to be performed and the criteria to be met.

. 227.13

(c) . . . dredged material can be considered to be environmentally acceptable for ocean dumping only under the following conditions: (1) The material is in compliance with the requirements of . 227.6; and

(2)(i) All major constituents of the liquid phase are in compliance with the applicable marine WQC after allowance for initial mixing; or (ii) When the liquid phase contains major constituents not included in the applicable marine WQC, or there is reason to suspect synergistic effects of certain contaminants, bioassays on the liquid phase of the dredged material show that it can be discharged so as not to exceed the limiting permissible concentration as defined in paragraph (a) of . 227.27. . . (3)(d) For the

purposes of paragraph (c)(2) of this section, major constituents to be analyzed in the liquid phase are those deemed critical by the District Engineer, after evaluating and considering any comments received from the Regional Administrator, and considering known sources of discharges in the area. In Step 2, the numerical mixing model (Appendix B) is run with chemical data obtained from an elutriate test conducted on the dredged material. The standard elutriate analysis is described in Section 10.1.2.1 and the analytical procedures for measuring constituents in the water are presented in Section 9.4.2. The modeling is, in effect, using data that more accurately represent the contaminant concentrations that will be present in the water column at the disposal site. If the numerical model (Appendix B) predicts that the concentration of all contaminants of concern in the water column are less than the applicable WQC and if no synergistic effects are suspected, the dredged material meets the LPC for the water column. If the model run shows that the WQC are exceeded, the LPC for the water column is not met.

5.1.3 Water-Column Toxicity Compliance

At present, there is no procedure to assess LPC compliance for water-column toxicity in Tier II for dredged-material contaminants without WQC or from effects of synergistic reactions (Figure 3-2). If WQC have not been established for all contaminants of concern or if synergistic effects are expected, further testing in Tier III is required to determine water-column LPC compliance. Consequently, toxicity evaluations and LPC determinations for these situations must take place in Tier III or IV. As a rule, *synergistic effects are to be suspected wherever there is more than one contaminant present in the sediment.*

In Tier II, one of three possible conclusions is reached regarding the toxicity of the proposed dredged material.

- Concentrations of all of the dissolved contaminants of concern in the dredged material, after allowance for initial mixing, do not exceed the applicable marine WQC beyond the boundaries of the disposal site at any time nor exceed the WQC anywhere in the marine environment 4 h after dumping. Additionally, synergistic effects from more than one contaminant of concern are not anticipated. Therefore , the dredged material complies with applicable WQC requirements of paragraph 227.13(c)(2)(i) and the LPC requirements for the water column of paragraph 227.13(c)(2)(ii). If so, no further information is necessary to determine compliance with the regulations regarding water-column impact, but benthic impact has to be evaluated. If the information warrants, it is acceptable to determine compliance with water-column effects criteria of paragraphs 227.13(c)(2)(i) and 227.13(c)(2)(ii) at Tier II and determine compliance with benthic effects criteria at another tier.
- The WQC requirements are met but one or more of the contaminants of concern do not have established marine WQC and/or synergistic effects of the contaminants are suspected. Therefore, determination of compliance with water-column effects criteria is not possible and water-column toxicity must be evaluated in Tier III or IV.

- Concentrations of one or more of the dissolved contaminants of concern, after allowance for initial mixing, exceed applicable marine WQC beyond the boundaries of the disposal site or exceed marine WQC within the site after the first 4 h. In this case, the dredged material does not comply with the WQC requirements of paragraph 227.13(c)(2)(i) and the LPC is exceeded.

5.2 BENTHIC IMPACT

As discussed above, the currently available Tier II procedure for evaluating potential benthic impact consists of evaluating the TBP. The TBP is calculated according to the guidance in Section 10.2. At present, this calculation can be performed for nonpolar organic compounds, but not for polar organic compounds, organometals, or metals. If such constituents are contaminants of concern in a dredged material requiring bioaccumulation evaluation, that evaluation has to take place in Tiers III and/or IV.

In the Tier II benthic-impact evaluation, a comparison is made between TBP calculated for the nonpolar organic contaminants of concern in dredged material and for the same constituents in the reference sediment. *If all the contaminants of concern in the dredged material are nonpolar organics,* one of the following conclusions is reached based on this comparison:

- The TBP for the nonpolar organic contaminants of concern in the dredged material does not exceed the TBP for the reference sediment and, therefore, the dredged material complies with bioaccumulation aspects of the benthic criteria in paragraph 227.13(c)(3). If so, no further information is necessary to determine compliance with the bioaccumulation regulations, but biological effects also have to be considered to determine compliance with the benthic criteria in paragraph 227.13(c)(3) (Appendix A). If the information warrants, it is acceptable to determine compliance with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) at Tier II, and determine compliance with the biological-effects aspects of the benthic criteria at another tier. Potential water-column impact also has to be considered.
- The TBP for the contaminants of concern in the dredged material exceeds the TBP for the reference sediment. In this case, the information is not sufficient to determine whether the dredged material complies with the bioaccumulation aspects of the benthic criteria in paragraph 227.13(c)(3), and further evaluation of bioaccumulation in Tiers III and/or IV is appropriate. Potential water-column impact also has to be considered.

Although the calculation of TBP is used to evaluate nonpolar organic compounds in Tier II, a particular dredged material may contain contaminants of concern for which it may be inappropriate to make this calculation. For these contaminants, bioaccumulation has to be evaluated in Tiers III and/or IV. However, even if the dredged material contains other contaminants of concern in addition to nonpolar organic contaminants of concern, it is still useful to calculate the TBP. The TBP provides an indication of the magnitude of

bioaccumulation of nonpolar organics that may be encountered in Tiers III and/or IV testing. Additionally, if the TBP of the nonpolar organics meets the decision guidance in this section, the calculation may eliminate the need for further evaluation of these compounds and thereby reduce efforts in Tiers III and/or IV.

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6.0 TIER III EVALUATION

Tier III testing assesses the impact of contaminants in the dredged material on appropriate sensitive organisms to determine if there is potential for the dredged material to have an unacceptable impact. The Tier III assessment methods are bioassays and bioaccumulation tests (Figures 3-1 through 3-3). When sublethal chronic-effects tests are developed and approved by EPA and the USACE, they will be included in this tier.

Tier III bioassays use lethality as the endpoint because lethality is easily interpreted and quantified. The bioassays are acute tests using organisms representative of the water-column and benthic environments at the disposal site. The recommended procedures for water-column bioassays (Figure 3-2) use appropriate sensitive marine water-column organisms (Section 11.1.1, Table 11-1). The assay for benthic impact (Figure 3-3) uses deposited sediment and appropriately sensitive benthic marine organisms (Section 11.2.1, Table 11-2).

Bioaccumulation also has to be considered to fully evaluate potential benthic impact (Figure 3-3). The results of bioaccumulation tests are used to predict the potential for uptake of dredged-material contaminants by organisms (Biddinger and Gloss, 1984; Kay, 1984). These tests may be conducted in the laboratory (Section 12.1). The Tier III information is usually sufficient for decision-making, or it may, in rare cases, indicate that further information on toxicity or bioaccumulation (or both) is required at Tier IV.

6.1 WATER-COLUMN BIOASSAYS

If additional water-column testing has been shown to be necessary (Section 5.1), the Tier III water-column evaluation (Figure 3-2) considers the effects, after allowance for initial mixing, of dissolved contaminants plus those associated with suspended particulates on water-column organisms. According to paragraph 227.13(c)(2)(ii) of the regulations (Appendix A), water-column bioassays must be used when there are not applicable marine water-quality criteria (WQC) for all the contaminants of concern or when there is reason to suspect the synergistic effects of certain contaminants. The bioassay and initial-mixing data results are generated as described in Section 11.1. The limiting permissible concentration (LPC) is defined in paragraph 227.27(a)(2) (Appendix A) as

> *That concentration of waste or dredged material in the receiving water which, after allowance for initial mixing, as specified in . 227.29, will not exceed a toxicity threshold defined as 0.01 of a concentration shown to be acutely toxic to appropriate sensitive marine organisms in a bioassay carried out in accordance with approved EPA procedures.*

After considering this requirement, one of the following conclusions is reached.

- The concentration of dissolved plus suspended contaminants, after allowance for initial mixing, does not exceed 0.01 of the acutely toxic concentration beyond the boundaries of the disposal site within the first 4 h after dumping or at any point in the marine environment after the first 4 h. Therefore, the dredged material complies with the water-column toxicity criteria of paragraphs 227.13(c)(2)(ii) and 227.13(c)(3) (Appendix A). If so, no further information is necessary to determine compliance with the regulations regarding water-column impact, but benthic impact has to be considered. If the information warrants, it is acceptable to determine compliance with the water-column effects criteria of paragraphs 227.13(c)(2)(ii) and 227.13(c)(3) at Tier III and determine compliance with the benthic effects criteria at another tier.
- The concentration of dissolved plus suspended contaminants, exceeds 0.01 of the acutely toxic concentration beyond the boundaries of the disposal site at any time and/or within the disposal site after the 4-h initial-mixing period. Therefore, the dredged material does not meet the water-column LPC as defined in paragraph 227.13(c)(2)(ii) or in paragraph 227.13(c)(3) (Appendix A).

6.2 WHOLE-SEDIMENT BIOASSAYS

Evaluation of benthic bioassays in Tier III (Figure 3-3) is based on data generated according to the guidance in Section 11.2. For benthic-effects evaluation, the LPC of the solid phase of dredged material is applicable and is defined in paragraph 227.27(b) (Appendix A) as

> *. . . that concentration which will not cause unreasonable acute or chronic toxicity or sublethal adverse effects based on bioassay results using . . . appropriate sensitive benthic marine organisms . . .*

.

Dredged material does not meet the LPC for benthic toxicity when bioassay organism mortality (1) is statistically greater than in the reference sediment and (2) exceeds mortality in the reference sediment by at least 10%. (or a value that is in accordance with approved testing methods, e.g., 20% for amphipod bioassays). The 10% value should be used unless another value is approved for use. If values other than 10% are to be used, they should be derived for each test species and test endpoint. The data supporting the values should meet quality-assurance (QA) standards and provide an adequate basis for regulation.

After considering this guidance, one of the following conclusions is reached for the acute toxicity of contaminants in the dredged material in Tier III.

- Mortality in the dredged material is not statistically greater than in the reference sediment, or does not exceed mortality in the reference sediment by at least 10%. Therefore, the dredged material meets the LPC for benthic toxicity and complies with the benthic bioassay criteria of paragraph 227.13(c)(3) (Appendix A). If so,

no further information is necessary to determine compliance with the LPC for benthic toxicity, but bioaccumulation also has to be considered under paragraph 227.13(c)(3). If the information warrants, it is acceptable to determine compliance with the benthic-bioassay criteria of paragraph 227.13(c)(3) at Tier III and with the bioaccumulation criteria of paragraph 227.13(c)(3) at another tier. Potential water-column impact also has to be considered.

- Mortality in the dredged material is statistically greater than in the reference sediment and exceeds the mortality in the reference sediment by at least 10%.* In this case, the dredged material exceeds the LPC and does not comply with the benthic bioassay criteria of paragraph 227.13(c)(3) (Appendix A).

6.3 BIOACCUMULATION BY BENTHOS

Bioaccumulation potential, as well as toxicity, has to be in compliance with the regulations before a dredged material can be considered acceptable for ocean dumping. The Tier III benthic-bioaccumulation tests provide for the determination of bioavailability through 10-day exposure tests if all contaminants of concern are metals or 28-day exposure tests if any contaminants of concern are organic or organometallic compounds. Information for evaluating bioaccumulation potential in Tier III for each of the contaminants of concern is presented in Section 12.1. Identification of the specific contaminants of concern in each dredged material is discussed in Section 4.2.

Bioaccumulation of most compounds, if it occurs, will be detectable after the Tier III 10- or 28-day exposure period, even though the steady state may not have been reached. Thus, while the Tier III tests may not determine steady-state bioaccumulation, they provide useful information about the potential for bioaccumulation (i.e., bioavailability).

Concentrations of contaminants of concern in tissues of benthic organisms following 10- or 28-day exposure to the dredged material are compared initially against applicable Food and Drug Administration (FDA) Action Levels for Poisonous or Deleterious Substances in Fish and Shellfish for Human Food, when such levels (i.e., limits) have been set for the contaminants. These action levels are the limits above which the FDA can take legal action to remove products from the market. The levels, which are based on human-health as well as economic considerations, are revised according to the criteria specified in 21 CFR 109 and 509. They do not include the potential for environmental impact on the contaminated organisms or on their nonhuman predators. The current FDA action levels are listed in Table 6-1. Updated lists may be obtained from the Food and Drug Administration, Center for Food Safety and Applied Nutrition, Industry Programs Branch, Bureau of Foods (HFF-326) 200 C Street S.W., Washington DC 20204; (202) 485-0020.

Because contamination of seafood in excess of FDA levels is considered a threat to human health, the guidance in this manual is that concentrations in excess of FDA levels in any test species may be considered unacceptable. This guidance applies even though

the test species may not be a typical human food item because contaminants can be transferred through aquatic food webs, and uptake to FDA levels in one species indicates the potential for accumulation in other species. FDA action levels do not consider ecological impact; however, for the purposes of this manual, they serve as an upper limit of acceptability.

Based on the comparison against FDA levels, one of the following conclusions is reached.

- Tissue concentrations of one or more contaminants of concern are statistically greater than applicable FDA action levels. Therefore, the dredged material exceeds the limiting permissible concentration (LPC) for bioaccumulation and does not comply with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A).
- Tissue concentrations of all contaminants of concern either are not statistically greater than applicable FDA action levels or there are no FDA levels for the contaminants of concern. In this case, the information is insufficient to determine compliance with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A), and the dredged material has to be further evaluated in Tier III as described below for bioaccumulation potential before a decision can be made.

Concentrations of contaminants determined in tissues of organisms following the 10- or 28-day exposure to dredged material and less than FDA action levels or in

the absence of FDA levels are compared to contaminant concentrations in tissues of organisms similarly exposed to reference sediment. One of the following conclusions is reached based on this comparison.

- Tissue concentrations of contaminants of concern in organisms exposed to dredged material do not statistically exceed those of organisms exposed to the reference sediment, and therefore the dredged material meets the LPC for bioaccumulation and complies with the benthic criteria of paragraph 227.13(c)(3) (Appendix A). If so, no further information is necessary to determine compliance with bioaccumulation regulations, but benthic-toxicity effects also have to be considered to determine compliance with the benthic criteria of paragraph 227.13(c)(3). Potential water-column impact also has to be considered.

- Tissue concentrations of contaminants of concern in organisms exposed to dredged material statistically exceed those of organisms exposed to the reference material. In this case, it is recommended that the EPA Regional Administrator and the USACE District Engineer develop and agree upon case-specific evaluative criteria, based on technical evaluations made with local input, that emphasize the various factors deemed appropriate in each area for determining compliance with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A).

To determine compliance with paragraph 227.13(c)(3), when the bioaccumulation of

contaminants in dredged-material tests statistically exceeds that in the reference-material tests, the following factors should be assessed to evaluate LPC compliance.

- Number of species in which bioaccumulation from the dredged material is statistically greater than bioaccumulation from the reference material
- Number of contaminants for which bioaccumulation from the dredged material is statistically greater than bioaccumulation from the reference material
- Magnitude by which bioaccumulation from the dredged material exceeds bioaccumulation from the reference material
- Toxicological importance of the contaminants whose bioaccumulation from the dredged material statistically exceeds that from the reference material
- Phylogenetic diversity of the species in which bioaccumulation from the dredged material statistically exceeds bioaccumulation from the reference material
- Propensity for the contaminants with statistically significant bioaccumulation to biomagnify within aquatic food webs (Biddinger and Gloss, 1984; Kay, 1984)
- Magnitude of toxicity and number and phylogenetic diversity of species exhibiting greater mortality in the dredged material than in the reference material
- Magnitude by which contaminants whose bioaccumulation from the dredged material exceeds that from the reference material also exceed the concentrations found in comparable species living in the vicinity of the proposed disposal site.

These and perhaps other factors are complexly interrelated; i.e., the acceptable level of each factor depends on its interaction with all other factors. These factors have to be considered in developing case-specific criteria (if needed) for dredged material assessed for bioaccumulation in the final step of Tier III. After considering these factors, one of the following decisions is reached.

- Dredged material meets the LPC for bioaccumulation and complies with the benthic criteria of paragraph 227.13(c)(3) (Appendix A). If so, no further information is necessary to determine compliance with bioaccumulation regulations, but toxicity and water-column effects also have to be considered to determine compliance with paragraph 227.13(c).
- Dredged material exceeds the LPC for bioaccumulation and does not comply with the benthic criteria of paragraph 227.13(c)(3) (Appendix A) and the LPC is not met.
- Information is insufficient to evaluate the LPC for bioaccumulation or to determine compliance with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A). Further evaluation of steady-state bioaccumulation in Tier IV is necessary to evaluate compliance.

6.4 REFERENCES

Kiddinger, G.R., and Gloss, S.P. 1984. The importance of trophic transfer in the bioaccumulation of chemical contaminants in aquatic ecosystems. Residue Rev. 91:104-130.

Kay, S.H. 1984. Potential for biomagnification of contaminants within marine and freshwater food webs. Tech. Rep. D-84-7, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

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7.0 TIER IV EVALUATION

Where a decision regarding toxicity or bioaccumulation has not been reached at earlier (i.e., lower-numbered) tiers or where circumstances warrant, Tier IV evaluations (Figure 3-1) are used to determine compliance with paragraph 227.13(c) (Appendix A). Tier IV tests consist of bioassays and bioaccumulation tests to determine the long-term effects of exposure to dredged material. Tier IV tests may be conducted for water-column evaluations (Figure 3-2) or benthic evaluations (Figure 3-3). In either case, Tier IV tests should be carefully selected to address the specific issues relevant to the case in question. Whatever the Tier IV test, the case-specific evaluative criteria for these tests have to be determined beforehand and agreed upon by EPA and the USACE, and have to be adequate to determine compliance with the requirements of paragraph 227.13(c).

7.1 BIOASSAYS

Tier IV bioassays should measure sensitive indicators of long-term effects of clear ecological importance, such as survival, reproduction, and, perhaps, the time to the onset of reproduction. Tier IV bioassays might be of longer duration than the Tier III tests, and might simulate the exposure conditions expected at the disposal site. Tier IV bioassays of deposited dredged material should maximize exposure to sediment-associated contaminants by focusing on infaunal organisms.

Because of the limited availability of appropriate and widely accepted procedures for Tier IV bioassays, these tests should be carefully selected to address the specific needs of each dredged-material disposal operation. Tier IV tests should be designed to provide more detailed information about the effects of exposure to the dredged material than does Tier III testing. Tier IV testing might be appropriate when the evidence is sufficient to require testing for carcinogens, mutagens, or teratogens under paragraph 227.13(c) of the regulations.

Tier IV allows generation of appropriate information about the proposed disposal operation when there is no other option for the generation of additional information. As discussed previously, even with the development of appropriate and acceptable new test procedures, including those for chronic exposure, it is anticipated that the case-by-case design and implementation of tests will continue to be a necessary component of Tier IV evaluations.

Case-specific evaluative criteria have to be developed for interpreting the results of Tier IV bioassays. These criteria have to be adequate to determine compliance with the requirements of paragraph 227.13(c) of the regulations.

7.2 BIOACCUMULATION BY BENTHOS

When a decision cannot be reached on the basis of the 10- or 28-day bioavailability data, it is appropriate to determine steady-state bioaccumulation of the contaminants of concern in Tier IV (Figure 3-3). Tissue samples used for this evaluation may be collected in the field (Section 12.2.2) or be generated by laboratory exposure of test organisms to the dredged material (Section 12.2.1). As with the Tier III evaluation of bioavailability from the 10- or 28-day tests, the first step in the evaluation of steady-state bioaccumulation is the comparison of steady-state concentrations of contaminants of concern to Food and Drug Administration (FDA) Action Levels for Poisonous or Deleterious Substances in Fish and Shellfish for Human Food. Following this comparison, one of the following conclusions is reached.

- Tissue concentrations of one or more contaminants of concern are statistically greater than applicable FDA action levels. Therefore, the dredged material exceeds the limiting permissible concentration (LPC) for bioaccumulation and does not comply with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A).
- Tissue concentrations of all contaminants of concern either are not statistically greater than applicable FDA action levels or there are no FDA levels for the contaminants of concern. In this case, the information is insufficient to determine compliance with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A), and the dredged material has to be further evaluated in Tier III as described below for bioaccumulation potential before a decision can be made.

Steady-state tissue concentrations of contaminants of concern that do not statistically exceed FDA action levels are next compared to steady-state concentrations of these contaminants that were determined in organisms exposed to reference sediment. Based on this comparison, one of the following conclusions is reached.

- Steady-state concentrations in organisms exposed to dredged material are determined not to statistically exceed those of organisms exposed to reference sediment, and therefore the dredged material meets the LPC bioaccumulation and complies with the bioaccumulation aspects of the benthic criteria in paragraph 227.13(c)(3) (Appendix A). No further information is necessary to determine compliance with the bioaccumulation regulations; however, benthic toxicity effects also have to be considered to determine compliance with paragraph 227.13(c). Potential water-column effects also have to be considered.
- Steady-state concentrations in organisms exposed to dredged material statistically exceed those of organisms exposed to reference sediment. In this case, the information is insufficient to evaluate the LPC or to determine compliance with the benthic criteria of paragraph 227.13(c)(3) (Appendix A), and further evaluation of steady-state bioaccumulation in Tier IV is necessary.

Steady-state contaminant concentrations in tissue samples that exceed those of organisms

exposed to reference sediment are compared against contaminant concentrations in field-collected benthic organisms (Figure 3-3), as described in Section 12.2.2.4. Field-collected organisms (preferably the same species as those used for the laboratory analysis) are those collected in the vicinity of the proposed disposal site and provide an indication of the steady-state body burden of the contaminants of concern around the site. One of the following conclusions is reached.

- The steady-state bioaccumulation of contaminants of concern does not statistically exceed the concentration of these contaminants in field-collected organisms, and therefore the dredged material complies with the bioaccumulation aspects of the benthic criteria in paragraph 227.13(c)(3) (Appendix A). If so, the LPC for bioaccumulation is met and no further information is necessary to determine compliance with the bioaccumulation regulations, but benthic-toxicity effects must also be considered to determine compliance with paragraph 227.13(c). Potential water-column effects also have to be considered.
- The steady-state bioaccumulation of contaminants statistically exceeds that of the field organisms. In this case, it is desirable that the EPA Regional Administrator and the USACE District Engineer develop and agree upon case-specific evaluative criteria, based on technical evaluations made with local input, that emphasize the various factors deemed appropriate in each area for determining compliance with the benthic criteria of paragraph 227.13(c)(3) (Appendix A).

In evaluating bioaccumulation potential to determine compliance with paragraph 227.13(c) where the steady-state bioaccumulation of contaminants of concern exceeds that of the field organisms, concern over potential adverse impact increases in direct relation to the

- Number of species in which bioaccumulation from the dredged material is statistically greater than bioaccumulation from the reference material
- Number of contaminants for which bioaccumulation from the dredged material is statistically greater than bioaccumulation from the reference material
- Magnitude by which bioaccumulation from the dredged material exceeds bioaccumulation from the reference material
- Toxicological importance of the contaminants whose bioaccumulation from the dredged material statistically exceeds that from the reference material
- Phylogenetic diversity of the species in which bioaccumulation from the dredged material statistically exceeds bioaccumulation from the reference material
- Propensity for the contaminants with statistically significant bioaccumulation to biomagnify within aquatic food webs (Biddinger and Gloss, 1984; Kay, 1984)
- Magnitude of toxicity and number and phylogenetic diversity of species exhibiting greater mortality in the dredged material than in the reference material
- Magnitude by which contaminants whose bioaccumulation from the dredged material exceeds that from the reference material also exceeds the concentrations found in comparable species living in the vicinity of the proposed disposal site.

These and perhaps other factors are complexly interrelated; i.e., the acceptable level of each factor depends on its interaction with all other factors. These factors have to be considered in developing case-specific criteria (if needed) for dredged material assessed for bioaccumulation in the final step of Tier IV. After considering these factors, one of the following decisions is reached.

- The dredged material meets the LPC for bioaccumulation and complies with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A). If so, no further information is necessary to determine compliance with bioaccumulation regulations, but toxicity and water-column effects also have to be considered to determine compliance with paragraph 227.13(c).
- The dredged material exceeds the LPC for bioaccumulation and does not comply with the bioaccumulation aspects of the benthic criteria of paragraph 227.13(c)(3) (Appendix A).

7.3 REFERENCES

Biddinger, G.R., and Gloss, S.P. 1984. The importance of trophic transfer in the bioaccumulation of chemical contaminants in aquatic ecosystems. Residue Rev. 91:104-130.

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Part III. Data Generation

8.0 COLLECTION AND PRESERVATION OF SAMPLES

If it is determined that physical, chemical, and biological testing is necessary (certain dredging operations may require no sampling), samples of dredged material, reference sediment, control sediment, organisms, and water will need to be collected. These are used for chemical analysis, bioassays, and bioaccumulation tests. This Section provides guidance for the development of a sampling plan that will lead to the collection, preservation, and storage of representative sediment, water, and organism tissue samples so that the physical and chemical characteristics and potential toxicity and bioaccumulation of dredged material can be accurately assessed.

Sampling is the foundation upon which all testing rests. Therefore, regional guidance is important for developing project-specific sampling plans. There are so many case-specific factors that influence sampling needs that detailed guidance of National scope is impractical. Table 8-1 represents the type of samples that may be required to complete the evaluations of Tiers II, III, and IV. This manual provides general guidance on items of major importance to consider when designing a sampling plan. The guidance focuses on two aspects of sampling design. One aspect is directed toward the project managers and administrative personnel who determine what tests are to be run and where and how samples are to be collected, handled, and tested. The second aspect, discussed later in this Section, concerns the technical details of sample collection and preservation.

8.1 BACKGROUND FOR A SAMPLING PLAN

A well-designed sampling plan is essential when evaluating the potential impact of dredged material discharge upon the marine environment. Before any sampling is initiated, the sampling plan has to be tailored to meet clearly defined objectives for individual dredging operations. In designing a generalized sampling program, factors such as the availability and content of historical data, the degree of sediment heterogeneity, the number and geographical distribution of sample-collection sites, the procedures for collection, preservation, storage, and tracking of samples, and the necessity for adequate quality assurance and quality control have to be carefully considered. The magnitude of the dredging operation and its time and budgetary constraints should also be considered.

An acceptable sampling plan should be in place before sampling begins. An adequate amount of sediment and water should be collected to conduct planned evaluations. Careful consideration of maximum allowable and recommended holding times for

sediments as well as the exigencies of resampling should be given careful consideration.

The importance of sampling is underscored by the fact that any evaluation is only as complete and reliable as the sampling (and sample handling and storage) upon which it is based. Thus, inadequacies or biases in sampling will manifest themselves by limiting the accuracy and/or the appropriateness of the study results.

The objective is to obtain samples to characterize the dredging and reference-material area. Sample size should be small enough to be conveniently handled and transported but large enough to meet the requirements for all planned analyses. The quality of the information obtained through the testing process is impacted by the following three factors.

- Collecting representative samples
- Using appropriate sampling techniques
- Protecting or preserving the samples until they are tested.

Ideally, the importance of each of the three factors will be fully understood and appropriately implemented for each study. In practice, however, this is not always the case. There may be occasions when study needs, time, or other resource constraints will limit the amount of information that should or can be gathered. When this is the case, each of these factors has to be carefully considered in light of the specific study purposes when designing a sampling plan.

An important component of any field sampling program is a preproject meeting with all concerned personnel. Attendants may include management, field personnel, laboratory personnel, data management/analysis personnel, and representatives of the regulators and the dredging proponent. The purposes of the meeting include (1) defining the objectives of the sampling program and (2) ensuring communication among participating groups.

Samples are collected and tested or analyzed to gain information. To be most useful, the information generated through a sampling program has to be directed at a specific need. The purposes of defining the objectives of a sampling program should be to clarify the information needed and to match these needs with the specific tests that supply the required information.

The stated objectives of a testing program should be more specific than just stating, for example, "An environmental evaluation of a proposed dredged material disposal operation." Although an environmental assessment may be the overall objective, the objectives of the testing program should be stated as specific tasks, such as

- Compare one or more sites in the dredging area with the reference area
- Determine the kind and/or distribution of chemical contaminants in the sediments of a dredging area
- Determine potential sediment toxicity
- Determine bioaccumulation potential.

The more explicitly the goals of a testing program can be stated, the easier it will be to

design an appropriate sampling plan. When the sampling plan is completed, to select the appropriate methods of preservation, all sampling procedures should be clearly defined, sample volumes should be clearly established, all logistical concerns should be fully addressed, and target analytes should be identified to class of compound.

8.2 COMPONENTS OF A SAMPLING PLAN

A sampling plan that meets the stated objectives has to cover certain issues. The following steps are a guideline to ensure that all essential information is provided.

- Review the engineering specifications for the dredging operation, including the dimensions of the dredging area, the dredging depth(s), and the volume of sediment for disposal.
- Evaluate the prior history and the existing database for the area. Identify relevant data and the need for additional data. Identify areas of potential environmental concern within the confines of the dredging operation.
- If appropriate, subdivide the dredging area into project segments on the basis of an assessment of level of environmental concern within the dredging area. This may be an iterative process that starts before sampling, using available information, and that is refined after sampling, based on new data.
- Determine the number of samples to be collected and select sampling locations. Choose methods and equipment for positioning vessels at established stations.
- Determine what sampling methods will be used.
- Define procedures for sample handling, preservation, and storage.
- Identify potential logistical problems and define safety precautions.
- Prepare a quality assurance/quality control plan.

The subsections that follow discuss each of these steps and provide general guidance for their conduct. Supplemental guidance on basic sampling considerations generally applicable to dredged material is discussed from a quality assurance perspective by EPA (1987).

8.2.1 Review of Dredging Specifications

A review of the engineering specifications for the dredging operation provides a general overview to serve as a basis for designing a sampling plan. The volume of material to be dredged and the method of dredging are two of several important factors used to determine the number of samples required. Knowledge of the thickness and physical characteristics of the material to be dredged will help to determine the kind of sampling equipment that is required. The boundaries of the dredging area have to be known to ensure that the number and location of samples are appropriate.

8.2.2 Historical Data

In developing a sampling plan, it is important to review all information relevant to the dredging site. Using pertinent available information to determine project segments and station locations within the dredging area can produce significant cost savings over researching for new data. Reviewing historical data is the first step in determining whether sediment might be contaminated. If the review identifies possible point sources of contamination, skewing the sampling effort toward these areas may be justified for thorough characterization of the potentially contaminated areas. On the other hand, increasing the proportion of samples in contaminated areas relative to other areas may lead to the conclusion that the socalled average contamination is higher than purported. To reduce problems in areas of unequally distributed contamination, the total sampling effort should be increased. The information gathered for the Tier I evaluation (discussed in Section 4.1) should be reviewed for assistance in designing the sampling plan.

A review of historical information should include the following.

- **Geotechnical, geochemical, and hydrodynamic data**

  The grain size, specific density, water content, and identification of sediment horizons are helpful in making operational decisions. Areas of high tidal currents and high wave energy tend to have larger grain-sized sediments than do quieter areas. Contaminants have a greater affinity for clay and silt than for sand. The available data should be consulted to examine the horizontal and vertical particle-size distribution.

- **Quality and age of available data**

  The value of the available data should be critically weighed. Existing high-quality data might lower costs by reducing the number of analytes measured or tests required for the proposed dredging operation. Even data that do not meet all current quality- assurance standards can sometimes provide useful general information about the operation. For example, there may have been significant improvements in sampling and analytical methods since the original study, or the original chain-of-custody or documentation procedures may have been inadequate. Information from such studies might be helpful in identifying areas of contamination, but not in accurately assessing the degree of contamination.

- **Spill data**

  Evidence of a contaminant spill within or near the area of the dredging may be an important consideration in identifying areas for sampling.

- **Dredging history**

  Knowledge of prior dredging may dramatically affect sampling plans. If the area is frequently dredged (every 1-2 years) or if the sediments are subject to frequent mixing by wave action or ship traffic, the sediments are likely to be relatively homogenous. Assuming that there is no major contaminant input, the sampling effort may be minimal. However, if there is information regarding possible contamination, a more extensive sampling effort may be indicated. New

excavations of material unaffected by anthropogenic input may require less intensive sampling for contaminants than does maintenance dredging.

8.2.3 Subdivision of Dredging Area

Sediment characteristics are likely to vary substantially within the limits of the area to be dredged as a result of geographical and hydrological features in the area. Areas of low hydraulic energy will be characterized by fine sediments that have a greater tendency to accumulate contaminants than do coarser-grained sediments. Sediments in heavily urbanized or industrialized areas are more likely to accumulate contaminants than do sediments farther removed from direct contaminant input.

Many dredging operations can be subdivided into project segments for sampling. A project segment is an area expected to have relatively consistent characteristics that differ substantially from the characteristics of adjacent segments. Project segments may be sampled with various intensities, and, if warranted by objectives of the study and test results, the dredged material from various project segments can be managed in different manners during dredging and disposal to limit environmental impact. When the sampling plan is developed, project segments can be designated, based on historical data, sediment characteristics, geographical configuration, depth of cut, sampling- or dredging-equipment limitations, results of pilot studies, known or suspected contaminant concentrations, etc. Surface sediments might be considered as a project segment that is separate from subsurface sediments at the same location if vertical stratification of contamination is expected. Large dredging operations located within industrialized areas might require subdivision into several project segments horizontally and into one or more segments vertically. A dredging operation characterized by relatively uniform distribution of sediment type in a nonindustrialized location might be considered as a single project segment. Vertical subdivisions usually are not appropriate in areas of rapid shoaling or in areas of high sediment mixing by ship scour. These areas are likely to be relatively homogenous vertically. Vertical subdivisions smaller than about 2-3 ft are impractical because a dredge operator cannot reliably control excavation with any finer precision. If analytical data or test results for two or more project segments prove to be similar, these segments should be treated as one large segment when considering disposal options. If the analytical and test results demonstrate important differences between project segments, an alternative disposal option may be necessary for a portion of the total sediment volume.

Any established sampling program should be sufficiently flexible to allow changes based on field observations. Certain characteristics of the sediments, such as color or texture, can be an indication of patchiness to the field crew chief. The greater the patchiness, the larger the number of samples that will be required to define the area. The project manager can refine a sampling program based on historical data and/or a preliminary sampling survey of the dredging area.

8.2.4 Selection of Sampling Sites and Number of Samples

The method of dredging, the volume of sediment to be removed, and the horizontal and vertical heterogeneity of the sediment are key to determining station locations and the number of samples to be collected for the total dredging operation and for each project segment. When appropriate to testing objectives, samples may be composited prior to analysis (with attention to the discussion later in this Section). The appropriate number of samples and the proper use of compositing have to be determined for each operation on a case-by-case basis.

The following factors should be considered in sampling-site selection.

- Objectives of the testing program
- Accessibility
- Flows
- Mixing
- Source locations
- Available personnel and facilities
- Other physical characteristics.

The actual sampling pattern to be used is, by necessity, dependent on the site because major point sources, land-use activities, hydrologic conditions, and sample variability fluctuate from area to area.

The pattern should consider contaminant sources in each project segment and currents that could be critical to the pattern of sediment distribution. Station locations within the dredging area should include areas downstream from major point sources and in quiescent areas, such as turning basins, side channels, and inside channel bends, where fine-grained sediments are most likely to settle. Project segments selected on the basis of suspected high contamination cannot be considered as representative of the contaminant distribution in the entire dredging area. Therefore, project segments representing the proportion of the overall dredging area expected to be less contaminated than other segments have to be sampled representatively also.

Several characteristics have been established to help to define the representativeness of a sample:

- The project segment being sampled is clearly defined.
- The sampling locations are distributed randomly within each project segment.
- More than one sample should be collected from each sampling location if sample variability is suspected.
- When sediment variability is unknown, it may be necessary to conduct a preliminary survey of the dredging area to better define the final sampling program.

Sediment composition can vary in the vertical dimension as well as in the horizontal dimension. Thus, samples should be collected over the entire depth that is to be excavated unless the sediments are known to be vertically homogenous or there are adequate data to demonstrate that the contamination does not extend throughout the depth to be excavated.

The easiest task in establishing a sampling program is to locate the areas of maximum concentration that generally are found near the major sources or areas of sediment deposition. However, the results from these sampling locations may not represent the range of concentrations in the total dredging area. Therefore, additional sampling has to be conducted in any areas for which inadequate data are available.

In relation to sample representativeness, it is possible to define two populations: (1) the actual composition of the area and (2) the composition of the samples obtained from the area. Ideally, these populations would be the same. However, in practice, there often are differences due to bias in the sampling program. Many factors contribute to bias, including disproportionate intensity of sampling in different parts of the dredging area and equipment limitations (i.e., extrapolating surface grab sample results to subsurface sediments).

It may be useful to develop a sampling grid for each project segment. The horizontal dimensions of each project segment are subdivided into grid cells of equal size; these are numbered sequentially within each project segment. Cells are then randomly selected for sampling. It may be important to collect more than the minimum number of samples required, especially in areas suspected of having high or highly variable contamination. Extra samples may be collected and archived should reexamination of a particular project segment(s) be warranted.

In some cases, it may be advisable to consider varying the level of sampling effort for separate project segments. Project segments suspected of containing environmentally important contaminants should be targeted for an increased level of effort so that the boundaries and characteristics of the contamination can be identified. A weighting approach can be applied whereby project segments are ranked in increasing order of concern. The weights can be used as factors when determining the number of samples within each project segment relative to other project segments.

One of the more important tasks is to determine the number of samples that should be collected within each project segment. In general, the number of samples required is inversely proportional to the amount of known information and is proportional to the level of confidence that is desired in the results and the suspected level of contamination. No specific guidance can be provided, but several general concepts are presented: (1) the greater the number of samples collected, the better the area will be defined; (2) the means of several measurements at each station within a project segment generally are less variable than individual measurements at each station would be; (3) statistics require replication because single measurements are inadequate to describe variability; and (4) the necessary number of samples is proportional to the heterogeneity of the sediment and the statistical power desired in the tests based on the sampling.

In all cases, the goal is to obtain sufficient information to evaluate the environmental impact of a dredging operation within the constraints of the operation. Although such constraints do not justify inadequate environmental evaluation, the reality of time and funding constraints have to be recognized. Possible responses to such constraints have been discussed by Higgins (1988). If the original sampling design does not seem to fit

time or funding constraints, several options are available:

- **Reduce the number of replicates at each station.**

  This provides a more synoptic survey of distribution patterns in the project segment, but makes statistical comparisons of individual stations less powerful. This may be the easiest approach, but is not necessarily the most desirable.

- **Maintain replicates, but reduce the number of sampling stations.**

  This results in less detailed definition of the project segment, but maintains the power of station-to-station comparisons.

- **Reduce the number of project segments into which the project is divided, but maintain the same total number of samples.**

  This also results in less detailed definition of each project segment, but maintains the power of station-to-station comparisons.

- **Maintain (or even increase) the number of stations sampled, and composite multiple samples from within a project segment so that a lower number of analyses are performed per project segment.**

Regardless of the final decision on project segments and the number of sample stations and replicates per project segment, stations within each segment should be randomly distributed. Expected degree of contamination will be the dominant factor in initially describing the proposed project segments. If there are likely to be important variables in potential dredged-material impact within a project segment, it may be advisable either to use a stratified random-sampling approach or to redefine project-segment boundaries. Once the data from the sampling are available, to maximize the homogeneity within segments, it may be advisable to redefine the boundaries of the project segments to be used in the actual dredging.

In decisions regarding compositing of samples, the objective of obtaining an accurate representation and definition of the dredging area has to be satisfied. Compositing provides a way to analyze sediments from more stations at the same cost or from the same number of stations at lower cost. However, compositing results in a less detailed description of the area sampled than would individual analysis of each station. If, for example, five analyses can be performed to characterize a project segment, the increased coverage afforded by collecting 15 individual samples and combining sets of three into five composite samples for analysis may justify the increased time and cost of collecting the extra 10 samples. Compositing can provide the large sample volumes required for some biological tests. Composite samples represent the socalled "average" of the characteristics of the individual samples making up the composite, and can closely represent the overall characteristics of the entire volume of the material to be dredged.

When a sediment sample is collected in the field, a decision has to be made as to whether the entire sediment volume is to be considered as the sample or whether the sediment volume represents separate samples (i.e., based on observed stratification, the top 2-3 ft of a core might be considered to be a separate sample from the remainder of the core).

After the sediment to be considered as a sample is identified, it has to be thoroughly homogenized. Core samples should be split before compositing. One half of the original sediment is archived should later analysis of the individual sample be required; the other half is combined with parts of other samples. These are thoroughly homogenized, producing the composite sample.

8.2.5 Sample-Collection Methods

Sample collection requires an experienced crew, an adequate vessel equipped with precise navigational equipment and winches, and noncontaminating sampling apparatus capable of obtaining relatively undisturbed and representative samples. The major sampling effort for a proposed dredging operation is oriented toward the collection of sediment samples for physical and chemical characterization or for biological tests. Collection of water samples might also be required to evaluate potential water-column impact. Collection of organisms near the disposal site might be necessary if there is a need to characterize indigenous populations at these locations or to assess concentrations of contaminants in tissues. Organisms for use in biological-effects and bioaccumulation tests may also be field-collected.

Guidance is provided in this Section regarding the selection and use of some equipment associated with sediment, water, or organism sampling. In general, a hierarchy for sample collection should be established to prevent contamination from the previous sample, especially when using the same sampling apparatus to collect samples for different analyses. At a station where water and sediment are to be collected, water samples should be collected prior to sediment samples. The vessel should be positioned downwind or downcurrent of the sampling device. When lowering sampling devices, care should be taken to avoid visible surface slicks. The deck and sample-handling area should be kept clean to help to reduce the possibility of contamination.

EPA (1987) provides useful sampling guidance from a quality-assurance viewpoint; this document may be followed on all points that are not in conflict with the guidance in this manual. Higgins and Lee (1987) provide perspective on sediment collection and analysis as commonly practiced in USACE Districts.

8.2.5.1 Sediment-Sample Collection

Sediment samples should be collected to the planned depth of excavation (including any "overdepth" dredging), unless the sediments are known to be vertically homogenous or the deepest sediments to be excavated are known to be uncontaminated. Care should be taken to avoid contamination of sediment samples during collection and handling. Samples designated for trace-metal analysis should not come into contact with metal surfaces, and samples designated for organic analysis should not come into contact with plastic surfaces. Samples for biological tests may be stored in clean polypropylene containers. Subsamples for particular groups of analytes may be removed from areas of the sample not in physical contact with the collecting instrument.

A coring device is recommended whenever sampling to depth is required. The choice of corer design depends upon the objectives of the sampling program, the sediment type, water depth, sediment depth, and currents. A gravity corer may be limited to cores of 1-2 m in depth, depending upon sediment grain size, degree of sediment compactness, and velocity of the drop. For penetration greater than 2 m, a vibratory corer or a piston corer may be preferable. The length of core that can be collected generally is limited to 10 core diameters in sand substrate and 20 core diameters in clay substrate. Longer cores can be obtained, but substantial sample disturbance results from internal friction between the sample and the core liner.

Freefall cores can cause compaction of the vertical structure of sediment samples. Therefore, if the vertical stratification in a core sample is of interest, a piston corer should be used. These devices utilize both gravity and hydrostatic pressure. As the cutting edge penetrates the sediments, an internal piston remains at the level of the sediment/water interface, preventing sediment compression and overcoming internal friction. If the samples will not be sectioned prior to analysis, compaction is not a problem, and freefall noncontaminating corers are a suitable alternative.

Corers are the samplers of preference in most cases because of the variation in contamination with depth that can occur in sediment deposits. Substantial variation with depth is unlikely in areas that have frequent ship traffic and from which sediments are dredged at short intervals. In these situations, accumulating sediments are resuspended and mixed semicontinu- ously by ship scour and turbulence, effectively preventing stratification. In such cases, grab samples can be representative of the mixed-sediment column, and corers should be necessary only if excavation of infrequently disturbed sediments below the mixed layer is planned.

Grab samplers are acceptable for collecting samples of reference or control sediments. A grab can be Teflon -coated to prevent potential contamination of trace-metal samples. The sampling device should be rinsed with clean water between samples.

8.2.5.2 Water-Sample Collection

If water samples are necessary, they should be collected with a noncontaminating pump or, if only a small volume of water is required, with a discrete collection bottle. When sampling with a pump, the potential for contamination can be minimized by using a peristaltic or a magnetically coupled impeller-design pump. The system should be flushed with the equivalent of 10 times the volume of the collection tubing. Also, any components within several meters of the sample intake should be noncontaminating (i.e., sheathed in polypropylene or be epoxy-coated). Concern must be exercised to limit potential sample contamination from research vessels and other apparatuses used in sampling.

A discrete water sampler should be of the close/open/close type so that only the target water sample comes into contact with internal sampler surfaces. Seals should be Teflon-coated whenever possible. Water-sampling devices should be acid-rinsed prior to

use for collection of trace-metal samples and rinsed with hexane (or other appropriate solvent) prior to collection of samples for organic analyses.

8.2.5.3 Organism Collection

If collection of epibenthic macrofauna is necessary, they may be collected with a trawl. Infaunal organisms may be collected with a benthic grab or a box corer. If organisms are to be maintained alive, they should be transferred immediately to containers with clean, well-oxygenated flowing seawater. Care should be taken to prevent organisms from coming into contact with potentially contaminated areas or fuels, oils, brass, copper, lead, galvanized metal, cast iron, or natural rubber.

8.2.6 Sample Handling, Preservation, and Storage

Detailed procedures for sampling handling, preservation, and storage should be part of the standard operating procedures (SOP) and protocols developed for each sampling operation. As samples are subject to chemical, biological, and physical changes as soon as they are collected, and unadulterated samples are necessary for an accurate evaluation of the dredged material. Sample handling, preservation, and storage techniques have to be designed to minimize any changes in composition of the sample by retarding chemical and/or biological activity and by avoiding contamination. Information regarding collection, volume requirements, container specifications, preservation techniques, and storage conditions for sediment, water, and tissue samples is discussed below and summarized in Table 8-2 *(25k)*. Additionally, EPA (1987) provides useful guidance on sampling quality assurance/quality control (QA/QC).

8.2.6.1 Sample Handling

Sufficient sample volume must be collected to

- Perform the necessary analyses
- Partition the samples for respective storage requirements (e.g., freezing for trace-metal analysis, refrigeration for bioassays)
- Archive portions of the sample for possible later analysis.

Sample handling is specific for each project and analyses to be conducted. Generally, samples to be analyzed for trace-metals should not come into contact with metals, and samples to be analyzed for organic compounds should not come into contact with plastics. All sample containers should be appropriately cleaned (acid-rinsed for analysis of metals; solvent-rinsed for analysis of organic compounds).

Samples should completely fill the storage container, leaving no airspace. If the sample is to be frozen, just enough air space should be allowed for expansion to take place. Container labels have to withstand soaking, drying, and freezing without becoming detached or illegible. The labeling system should be tested prior to use in the field.

Sediment samples for biological testing should have all living organisms removed from

the sediment prior to testing. This can be best accomplished by press-sieving the sediments through a 1-mm-mesh screen. Other matter retained on the screen with the organisms, such as shell fragments, gravel, and debris, should be recorded and discarded. Prior to use in bioassays, all sediments should be thoroughly homogenized.

8.2.6.2 Sample Preservation

Because the first few hours are the most critical to changes in the sample, preservation steps should be taken immediately upon sediment collection. There is no universal preservation or storage technique. A technique for one group of analyses may interfere with other analyses. This problem can be overcome by collecting sufficient sample volume to utilize specific preservation or storage techniques for specific analytes or tests. Preservation, whether by refrigeration, freezing, or addition of chemicals, should be accomplished onboard the collecting vessel whenever possible. If final preservation techniques cannot be implemented in the field, the sample should be temporarily preserved in a manner that retains the integrity of the sample. Onboard refrigeration is easily accomplished with coolers and ice; however, samples should be

segregated from melting ice or cooling water. Samples that are to be frozen on board may simply be placed in a cooler with dry ice. Sediment samples for biological analysis should be preserved at 4øC, never frozen or dried.

Additional guidance on sample preservation is given in Table 8-1.

8.2.6.3 Sample Storage

The elapsed time between sample collection and analysis should be as short as possible. The sample storage duration for chemical evaluations is specific to the chemical analyses to be conducted (Table 8-1). For biological testing, the samples *should* be tested within 2 weeks of collection, but the samples may be stored up to 6 weeks, if necessary. With passing time, moderately contaminated sediment in storage tends to become increasingly toxic to the test organisms. The longer the samples are stored, the more difficult it is to accurately determine LPC compliance.

8.2.7 Logistical Considerations and Safety Precautions

A number of frustrations in sample collection and handling can be minimized by carefully thinking through the process and requirements before going to the field. Well trained and experienced field crews should be used. Backup equipment and sampling gear and appropriate repair parts are advisable. A surplus of sampling containers and field data sheets should be available. Sufficient ice and adequate ice-chest capacity should be provided, and the necessity of replenishing ice before reaching the laboratory should be considered. A vessel with adequate deck space is safer and allows more efficient work than an overcrowded vessel. Unforeseeable circumstances are to be expected in field sampling, and time to adequately deal with the unforeseen has to be included in sampling schedules. Appropriate safety precautions have to be observed during field sampling

activities.

Samples have to be properly disposed when no longer needed. Ordinary sample- disposal methods are usually acceptable, and special precautions are seldom appropriate. According to the Characterization and Assessment Division of the EPA Office of Solid Waste and Emergency Response, under 40 CFR 261.4(d)(1) even the most contaminated samples, if collected for the sole purpose of testing, are not subject to requirements of the Federal hazardous-waste management regulations. In addition, under 40 CFR 261.5(a), if the waste generated is less than 100 kg per month, the generator is conditionally exempt as a small- quantity generator and may accumulate up to 1000 kg of waste on the property without being subject to the requirements of Federal hazardous-waste regulations. When samples have to be shipped, 49 CFR 100-177 should be consulted for current Department of Transportation regulations on packing and shipping.

8.2.8 Quality Control

Although Section 14 is devoted to QA/QC practices, it is appropriate at this point to discuss QA/QC issues specific to the collection and preservation of samples. An effective quality-control program has to be an integral part of a dredging evaluation from initiation of field collections. Potential for sample deterioration and/or contamination occurs during sample collection, handling, preservation, and storage. Approved protocols and standard operating procedures should be followed, and experienced personnel should be responsible for maintaining the integrity and identity of the samples from collection through laboratory analysis. EPA (1987) should be consulted for additional guidance generally appropriate to dredged material.

The following areas should receive special attention relative to quality control.

8.2.8.1 Documentation

A complete record of all field procedures should be maintained, including station locations, sampling methods, sample handling, preservation, and storage procedures. Dates and times of collection, preservation, and storage should be recorded. A sample-inventory log and a sample-tracking log should be maintained. Any circumstances potentially affecting sampling procedures should be documented.

8.2.8.2 Standard Operating Procedures

Written SOPs should be available for routine procedures performed during field collections. Personnel should be thoroughly familiar with these procedures before sampling is initiated.

8.2.8.3 Sample Labels

At a minimum, the following information should be included on a sample label.

- Unique identifying code

- Location (station number) and depth
- Analysis or test to be performed
- Preservation and/or storage method
- Date/time of collection
- Special remarks if appropriate
- Initials of person collecting the sample.

8.2.8.4 Sample Tracking

A procedure for tracking samples from collection through completion of analysis and sample disposal has to be in place. This procedure should incorporate a system for monitoring the condition of the sample during transport and storage. Appropriate personnel should be assigned responsibility for sample tracking and sample custody.

8.2.8.5 Archived Samples

A sample storage bank containing replicates or subsamples of analyzed samples or extra unanalyzed samples may be beneficial, especially if anomalous results are found from analyzed samples or if additional information or analyses are needed to better define sediment characteristics. Archived samples should be properly stored and inventoried.

8.3 REFERENCES

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9.0 PHYSICAL ANALYSIS OF SEDIMENTAND CHEMICAL ANALYSIS OF SEDIMENT, WATER, AND TISSUE SAMPLES

This Section provides guidance on the selection of chemical and physical parameters to aid in evaluating the acceptability of dredged material for proposed ocean disposal, and on the methods used to analyze these parameters.

The methods cited in this Section may be used to develop the required chemical information. However, other methods may provide similar results, and the final choice of analytical procedures depends upon the needs of each evaluation. In all cases, state-of-the-art methods should be used.

Any dredged material from estuarine or marine areas contains salt. The salt can interfere with the results obtained from some analytical methods. *Any methods proposed for the determination of parameters in sediment and water from estuarine or marine environments have to explicitly address steps taken to control salt interference.*

9.1 PHYSICAL ANALYSIS OF SEDIMENT

Ocean-dumping evaluations require that the physical characteristics of the dredged material be determined and used to help to assess the impact of dumping on the benthic environment and the water column. The physical analysis of sediment samples is the first step in the overall process of sediment characterization. Physical analysis provides general information on the physical characteristics of the dredged material and it can be used to assess the behavior of these sediments after disposal. These data are valuable also in helping to identify appropriate control and reference sediments for biological tests. In addition, the physical parameters can be helpful in evaluating the chemical measurements that are made as a later step in the characterization process.

The general analyses that are recommended are (1) grain size, (2) total solids/specific gravity.

Grain-size analysis is a measure of the frequency distribution of the size ranges of the particles that make up the sediment (Plumb, 1981; Folk, 1980). The general size classes of gravel, sand, silt, and clay are the most useful in describing the size distribution of particles in dredged-material samples.

Total solids is a gravimetric determination of the organic and inorganic material remaining in a sample after it has been dried at a specific temperature. The total-solids values generally are used to convert concentrations of the chemical parameters from a

wet-weight to a dry-weight basis. The specific gravity of a sample is the ratio of the mass of a given volume of material to an equal volume of distilled water at the same temperature (Plumb, 1981). Because the specific-gravity analysis requires a dry sample, it is performed usually in conjunction with the total-solids determination. The specific gravity of a dredged-material sample can be used to help to predict the dispersal and settling characteristics of dredged material upon ocean disposal.

Quality-control (QC) procedures for the general characterization of sediments are necessary to ensure that the data meet acceptable criteria for precision and accuracy. At a minimum, one triplicate analysis should be performed for every 20 samples analyzed, except for TOC where all samples should be run in triplicate. In addition, one procedural blank per 20 samples should be run and the results reported for TOC analysis. Standards used for TOC determinations have to be verified by using independent check standards to verify the accuracy of the results. Quality-control limits have to be agreed upon for each analytical procedure, and have to be consistent with the overall quality-assurance (QA) plan. Standard reference materials are not available for the determination of the physical parameters in sediments; however, where possible, laboratory standards should be analyzed with the same frequency as the triplicate analyses. QA is discussed in Section 14.

9.2 DETECTION LIMITS

The selection of appropriate method detection limits (MDL) is important. MDLs should be lower than the appropriate values against which the data are to be compared for interpretation. The detection limits for an analyte should be no greater than one-third (one-half log unit) of the appropriate value for the analyte and matrix of concern. An MDL of one-fifth to one-tenth the appropriate value is desirable and sufficient in most cases. This is necessary to evaluate whether the concentration of the analyte is approaching the value critical to the decision-making process.

Further, the MDL has to be sufficiently below the appropriate value so that there is a diminished variability in numerical values in the vicinity of the appropriate value. Since no conclusion can be more certain than the least-certain measurement, excessively low MDLs will not contribute to conclusions if sampling error is the dominant variable factor. For some contaminants, such as dioxin, every effort has to be made to achieve consistent quantitation at the lowest possible level. The detection limits have to be documented and reported for all analyses.

9.3 CHEMICAL ANALYSIS OF SEDIMENT

9.3.1 Selection of Analytical Targets (Sediment)

Chemical analysis provides information about the chemicals present in the dredged

material that, if biologically available, could cause toxicity and/or be bioaccumulated. This information is valuable for exposure assessment and for deciding which of the contaminants present in the dredged material to measure in tissue samples.

If the historical review conducted in Tier I (Section 4.1) fails to produce sufficient information to develop a suitable list of potential contaminants, a list of target chemicals has to be compiled.

There are many chemicals that could be included as target analytes. Target analytes should be selected from the priority pollutant list (Table 9-1 *(20k)*) and the information obtained from the historical review. In the context of the regulations, analysis of polynuclear aromatic

hydrocarbons (PAH) in dredged material should focus on those PAH compounds that are on the priority pollutant list (Clarke and Gibson, 1987). In addition, the target list should be expanded to include other contaminants that historical information or commercial and/or agricultural applications suggest could be present at a specific dredging site for example, dioxins where there have been industrial fires and tributyltin near ships on which these compounds have been used.

All sediments should be analyzed for total organic carbon (TOC). The TOC content of sediment is a measure of the total amount of oxidizable organic material in a sample. The TOC method should be based on high-temperature combustion rather than on chemical oxidation. Some classes of organic compounds are not fully degraded by chemical/ultraviolet techniques. The volatile and nonvolatile organic components make up the TOC of a sample. Because inorganic carbon (e.g., carbonates and bicarbonates) can be a significant proportion of the total carbon in some sediment, the sample has to be treated with acid to remove the inorganic carbon prior to TOC analysis. The method of Plumb (1981) recommends HCl as the acid. An alternative choice might be sulfuric acid since it is nonvolatile, is used as the preservative, and does not add to the chloride burden of the sample. Whatever acid is used, it has to be demonstrated on sodium chloride blanks that there is no interference generated from the combined action of acid and salt in the sample. The EPA Region II Laboratory at Edison, New Jersey, has also developed an acceptable method for TOC analysis. It is available from U.S. Environmental Protection Agency, Region II, Surveillance and Monitoring Branch, Woodbridge Avenue, Edison, NJ 08837.

9.3.2 Selection of Chemical Analytical Techniques (Sediments)

Once the list of target analytes for sediments has been established, the analytical methods for the analytes have to be determined. The methods will, to some degree, dictate the amount of sediment sample required for each analysis. Guidelines for the amount of sample to be collected are given in Table 9-2. These general sample sizes take into consideration the fact that more than one analysis may be required for each group of analytes. The amount of sample used in an analysis affects the detection limits attainable by a particular method.

For priority pollutants in sediments, the MDLs provided by EPA (1986a) may be used as general guidelines. These detection limits are analytical goals rather than requirements. Site- or operation-specific objectives may make lower or higher detection limits appropriate. If lower MDLs are required, the analysis may require more sensitive instrumentation, larger sample sizes, or additional cleanup/concentration steps. For most coastal sediments, suitable analytical methodology will control interferences such that required detection limits will be reached. A discussion of sediment MDL values is presented by Tetra Tech (1986a) and EPA (1986a). In any event, QC data should corroborate the detection limits reached, and any discrepancies have to be justified by the data.

The recommended method for the analysis of semivolatile and volatile priority pollutants in sediment is described by Tetra Tech (1986a). Analysis for organic compounds should always use capillary-column gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS) techniques. These methods provide analytically sound techniques that yield accurate data on the concentrations of chemicals in the sediment matrix. The analytical techniques for semivolatile organic compounds generally involve the solvent extraction of the organic constituents from the sediment matrix and subsequent analysis, after cleanup, using GC or GC/MS. The extensive cleanup is necessitated by the likelihood of (1) biological macromolecules, (2) sulfur from sediments with low or no oxygen, and (3) oil and/or grease in the sediment. The analysis of volatile organic compounds incorporates purge and trap techniques with analysis by either GC or GC/MS. If dioxin analysis is being performed, the methods of Kuehl *et al.* (1987) or Smith *et al.* (1984) should be consulted.

For many metals analyses, the concentration of salt may be much greater than the analyte of interest and cause unacceptable interferences in certain analytical techniques. In such cases, the freshwater approach of acid digestion followed by inductively coupled plasma or graphite furnace atomic absorption spectroscopy (GFAAS) needs to be coupled with appropriate techniques for controlling this interference. Further, it has to be remembered that Cr, Se, Sn, Sb, and As generally occur as cations with several possible oxidation states, whereas the elements Fe, Zn, Pb, Ni, Cd, and Cu occur as hydrated cations (also with different oxidation states possible). The Hg method shown by EPA (1986a) may be used for sediment analysis. Tributyltin may be analyzed by the method of Rice *et al.* (1987), and selenium and arsenic by the method of EPRI (1986).

The techniques for the analysis of chemical constituents have some inherent limitations for sediment samples. Interferences encountered as part of the sediment matrix, particularly in samples from heavily polluted areas, may limit the ability of a method to detect or quantify some analytes. Consequently, the most selective methods using GC/MS techniques are recommended for all nonchlorinated organic compounds because GC/MS analysis can often avoid problems due to matrix interferences. Gas chromatography/electron-capture detection (GC/ECD) methods are recommended as the primary analytical tool for all polychlorinated biphenyl (PCB) and pesticide analyses because GC/ECD analysis will result in lower detection limits. Two-column GC/ECD confirmation of all analytes is recommended. Alternatively, GC/MS using selected ion

monitoring (SIM) can be used for PCB and pesticide analysis. A total extraction of metal ions is not necessary. The standard aqua regia extraction yields consistent and reproducible results. A total extraction of the metals can be achieved only by acid fluoride or flux fusion methods.

The traditional methods for the analysis of PCB quantify PCB as aroclor mixtures, which can result in errors in determining concentrations (Brown *et al.,* 1984). The mixture of PCB congeners making up the aroclors changes due to physical, chemical, and/or biological processes altering the distribution of individual congeners in the environment after release. Techniques that rely on quantification of PCB by aroclor assume that the distributions of PCB

congeners found in environmental samples are identical to industrial formulations. This is not the case. In addition, aroclor determinations do not yield information on the potential biological significance of the PCBs (McFarland and Clarke, 1989). The most toxic PCB congeners lie mainly within the tetra-, penta-, and hexa-chlorobiphenyl isomer groups (McFarland *et al.,* 1986). More meaningful biological and toxicological information about PCB concentrations and more accurate analytical-chemistry data can be obtained by analyzing and quantifying PCBs as individual congeners or isomer classes (Cl1-Cl10). Total PCBs can be determined by the sum of the individual congeners. This summation more accurately represents the PCB concentration in samples, as shown in the National Oceanic and Atmospheric Administration Mussel Watch Program (NOAA, 1989). PCB congener analytical methods are recommended for all analyses of PCB in sediments. Table 9-3 *(15k)* lists the congeners recommended for analysis based on environmental abundance, persistence, and biological importance (McFarland and Clarke, 1989). The preparation for analysis should follow the techniques described by Tetra Tech (1986a) or EPA (1986a), but the instrumental analysis and quantification of the PCBs should be performed by using standard capillary GC columns, on individual PCB isomers according to the methods reported by NOAA (1989) (see also Stalling, 1987; Dunn, 1984; Schwartz, 1984; Mullin, 1984). Based on quantitation of the congeners listed in Table 9-3 *(15k)*, PCB concentrations should also be summed to give total PCBs in the sample according to the NOAA (1989) methods.

As stated earlier, the list of target analytes should include compounds that background and historical information suggest may be present. To further ensure that toxic compounds not included in the priority pollutant list are not overlooked in the chemical characterization of the dredged material, the analytical results should also be scrutinized by trained personnel for additional analytes that are not on the target list. The presence of persistent major socalled unknown analytes on gas chromatograms or reconstructed ion chromatograms should be noted. In such a case, methods involving GC/MS techniques for organic compounds are recommended for the identification of unknown chemicals.

9.3.3 Quality Control

Although Section 14 presents general QC/QA considerations, the EPA methods for the analysis of priority pollutants include detailed QC procedures and requirements that are

appropriate for discussion here. These guidelines should be followed rigorously throughout the chemical analysis. General QC procedures should include the analysis of a procedural blank and a matrix spike along with every 10 - 20 samples processed. To measure analytical precision, one sample should be analyzed in triplicate for every 10 - 20 samples analyzed. The standard deviation and coefficient of variation should be reported. In addition, recoveries of surrogate spikes should be documented and all analytical instruments calibrated at least daily. All calibration data should be submitted to the laboratory QA officer for review.

Standard reference materials (SRM), if available, should also be routinely analyzed to determine analytical accuracy. SRMs may be obtained from the organizations listed in Table 9-4. One SRM sample should be analyzed with every batch of 10 - 20 samples. Some samples of SRMs for organic analytes include National Research Council of Canada (NRC) marine sediment HS-1 and HS-2 for PCB; NRC marine sediment HS-3, HS-4, HS-5, and HS-6 for PAH; and National Institute for Standards and Technology (NIST) SRM #1647 and SRM #1597 for PAH. SRMs for metals analysis include NBS estuarine sediment (SRM #1646); NRC marine sediments MESS-1, BCSS-1, and PACS-1; and International Atomic Energy Agency (IAEA) marine sediment SD-N-1/2(TM). Since new SRMs are appearing constantly, current listings of appropriate agencies should be consulted frequently. The QA program has to document the ability of the selected methods to cope with the high salt content of sediments.

9.4 CHEMICAL ANALYSIS OF WATER

9.4.1 Recommended Analytical Targets (Water)

Analysis of seawater to determine the potential release of dissolved chemical constituents from the dredged material (standard elutriate) may be necessary to determine compliance with the regulations. Elutriate tests (Section 10.1.2.1) involve mixing dredged material with dredging-site water and allowing the mixture to settle. The portion of the dredged material that is considered to have the potential to impact the water column is the supernatant remaining after undisturbed settling. Chemical analysis of the elutriate allows a direct comparison of the data, after allowance for initial mixing, to applicable marine water-quality criteria (WQC). When collecting samples for elutriate testing, consideration should be given to the large volumes of water and sediment required to prepare triplicate samples for analysis. In some instances, when there is poor settling, the elutriate preparation has to be performed successively several times to accumulate enough water for testing.

In selecting target analytes for water analysis, historical water-quality information from the dredging site should be evaluated along with data obtained from the chemical analysis of sediment samples. The data from the chemical evaluation of the dredged material provide a known list of constituents that might affect the water column. All target analytes identified in the sediment chemical analysis should initially be considered

potential targets for water analysis. Nonpriority-pollutant chemical components that are found in measurable concentrations in the sediments should be included as targets for the water analysis if review of the literature indicates that these analytes have the potential to bioaccumulate in animals [i.e., have a high *K*ow or bioconcentration factor (BCF)] and are of toxicological concern.

9.4.2 Selection of Analytical Techniques (Water)

In contrast to freshwater, there are generally not EPA-approved methods for analysis of saline water. Application of the freshwater methods to seawater will frequently result in much higher MDLs than are common for freshwater unless care is taken to control the effects of salt on the analytical signal. It is therefore extremely important to ascertain a laboratory's ability to execute methods and attain acceptable MDLs in matrices containing up to 3% sodium chloride.

Once the list of target analytes for water is established, the methods for analysis should be selected. The water volume delivered to the laboratory for specific analytical methods may vary. A minimum of 1 L of elutriate should be delivered to the laboratory for metals analysis (as little as 100 mL may be analyzed). One liter of elutriate should be analyzed for organic compounds. For water samples from the dredging or disposal sites, 10-L water samples should be analyzed for organic analytes and 1-L water samples should be delivered for metals analysis. Additional water samples might be required for any supplemental target compounds that cannot be determined as part of the analyses for metal or organic priority pollutants. The size of the sample is one of the limiting factors in determining the detection limits for the water analyses. In some cases, the 10-L seawater volume for organic analysis will provide MDLs below the applicable marine WQC. MDLs for these water analyses should be established on the assumption that the seawater MDLs should be lower than the WQC concentrations. Laboratories participating in this program should routinely report MDLs achieved for a given analyte.

Many of the methods cited below for priority pollutants correspond to the methods established by EPA for freshwater analysis. Modifications or substitute methods (e.g., additional extract concentration steps, larger sample sizes, or concentration of extracts to smaller volumes) might be necessary to properly determine analyte concentration in seawater or to meet the desired MDLs.

Detailed methods for the analysis of organic and inorganic priority pollutants in water are referenced in the *Federal Register* (1984, Vol. 49, No. 209) and in Methods for the Chemical Analysis of Water and Wastes (EPA, 1982). Additional approved methods can be found in U.S. EPA Contract Laboratory Program Statement of Work for Organics Analysis, Multi-Media, Multi-Concentration (EPA, 1986b); Standard Methods for the Analysis of Water and Waste Water (APHA, 1989); Annual Book of Standards. Part 31, Water (ASTM, 1980); and Bioaccumulation Monitoring Guidance: 1. Estimating the Potential for Bioaccumulation of Priority Pollutants and 301(h) Pesticides Discharged into Marine and Estuarine Waters (Tetra Tech, 1985). Most of these methods will require modification to achieve low MDLs in seawater. Analysis of the semivolatile organic

priority pollutants involves a solvent extraction of water with an optional sample cleanup procedure and analysis using GC or GC/MS (Tetra Tech, 1986). The volatile priority pollutants are determined by using purge and trap techniques and are analyzed by either GC or GC/MS. If dioxin analysis is necessary, methods of Mehrle *et al.* (1988) should be consulted.

Other methods available for metals are: cadmium, copper, lead, iron, zinc, silver (Danielson *et al.,* 1978); arsenic (EPRI, 1986); selenium and antimony (Sturgeon *et al.,* 1985); very low levels of mercury (Bloom *et al.,* 1983); and tributyltin (Rice 1987).

A primary requirement of the analysis of seawater for inorganic and organic priority pollutants is to obtain detection limits that will result in usable, quantitative data that can subsequently be compared against applicable marine WQC to determine compliance with the limiting permissible concentration (LPC). Many existing EPA methods for freshwater analysis need to be adapted to achieve environmentally meaningful detection limits in seawater. Particularly of concern are procedural blanks and matrix interferences caused by the salt in seawater. Some modifications to the analytical methods for organic compounds might be required to sufficiently lower the MDLs. For example, it is recommended that sample extracts be concentrated to the lowest possible volume prior to instrumental analysis, and that instrumental injection volumes be increased to lower the limits of detection for the analytical methods used. All PCB and pesticide analytes should be analyzed by using GC/ECD, since the GC/ECD methods are more sensitive to these compounds and will lower the detection limits. PCB should be quantified as specific congeners (Mullin *et al.,* 1984; Stalling *et al.,* 1987) and as total PCBs based on the summation of particular congeners. Methods for specific PCB congener analysis are available from NOAA (1989). The congener method is accurate, provides lower detection limits, and is less subject to matrix interferences based on the selection of the individual PCB congeners used to quantify PCB.

The analysis of metals in seawater is subject to matrix interferences from sea salts, particularly sodium and chloride ions, when the samples are concentrated prior to instrumental analysis. The presence of salts in seawater samples might require the use of alternate analytical approaches to the EPA-approved freshwater methods to achieve the desired MDLs. The gold-amalgamation method with cold-vapor atomic absorption spectrophotometry (AAS) analysis is recommended to eliminate seawater matrix interferences for mercury analysis. Methods using solvent extraction and AAS analysis might be required to reduce seawater matrix interferences for the analysis of other target metals. Graphite-furnace AAS techniques after extraction are recommended for the analysis of metals, with the exception of mercury. Appropriate techniques should be used on the instruments to reduce salt interferences.

9.4.3 Quality Control

Section 14 presents a general discussion of appropriate QA/QC practices. The methods recommended for the analysis of priority pollutants in water include detailed QC procedures and requirements. These guidelines should be followed closely throughout the

chemical analyses. Minimum QC procedures should include the analysis of a procedural blank and a matrix spike along with every 10 - 20 samples processed. Triplicate analysis of one sample and analysis of appropriate SRMs should be conducted with the same frequency as the blanks and matrix spikes. SRMs for organic priority pollutants are not currently available for seawater, but reference materials for inorganic compounds may be obtained from the organizations listed in Table 9-4. Seawater matrix spikes of target analytes (e.g., seawater spiked with NIST SRM 1647 for PAH) should be used to fulfill analytical accuracy requirements. Some available SRMs for priority pollutant metals in seawater are NRC seawater CASS-1 and NRC seawater NASS-2.

Since many MDL goals might be well below what current freshwater methods are able to do, it is necessary that an appropriate part of the QA program require laboratories to establish their own MDLs and provide data to support their detection limits. It is also incumbent on participating laboratories to show that modifications made to existing methods are adequately precise, accurate, and free of salt interference from seawater.

9.5 CHEMICAL ANALYSIS OF TISSUES

9.5.1 Recommended Analytical Targets (Biota)

Bioaccumulation is evaluated by analyzing the tissue of the test organisms for contaminants that are selected from the list of target analytes as being of contaminants of concern for a specific dredged material. Sediment-chemistry data and available information on the bioaccumulation potential of those analytes has to be interpreted to establish which compounds are contaminants of concern in the tissues of biota.

The *n*-octanol/water partition coefficient (*K*ow) has traditionally been used to estimate the BCFs of many chemicals, including the priority pollutants, in organism/water systems (Chiou *et al.*, 1977; Kenaga and Goring, 1980; Veith *et al.*, 1980; Mackay, 1982).

When identifying organic contaminants of concern for bioaccumulation, a useful rule of thumb is that the potential for bioaccumulation increases as *K*ow increases. This general relationship is often true for compounds with log *K*ow less than approximately 6. Above this value, there is less of a tendency for bioaccumulation potential to increase with increasing *K*ow. Consequently, the relative potential for bioaccumulation of organic compounds can be estimated from the *K*ow of the compounds. EPA (1985) recommends that compounds for which the log *K*ow is greater than 3.5 be flagged for consideration for further evaluation of bioaccumulation potential. Based on the existing data, the organic compound classes of priority pollutants with the greatest potential to bioaccumulate are PAHs, PCBs, pesticides, and some phthalate esters. Generally, the volatile organic, phenol, and organonitrogen priority pollutants are not readily bioaccumulated. Some exceptions might be the chlorinated benzenes and the chlorinated phenols. Table 9-5 *(21k)* indicates the relative bioaccumulation potential of organic priority pollutants based on *K*ow. If PCBs or PAHs are identified for analysis in tissues, the guidance on selection

of specific analytical target compounds in Sections 9.3.1 and 9.3.2 should be followed.

The priority pollutant metals that might tend to bioaccumulate based on available BCF data are mercury, copper, arsenic, cadmium, zinc, lead, and chromium. Table 9-6 ranks the bioaccumulation potential of the priority pollutant metals based on calculated BCFs. Dredged- material contaminants with BCFs greater than 1000 (log BCF >3) should be further evaluated for bioaccumulation potential. Tables 9-5 and 9-6 have to be used with caution because they are based on calculated bioconcentration from water. Sediment-bioaccumulation tests, in contrast, are concerned with accumulation from a complex medium via all possible routes of uptake. The

appropriate use of the tables is to help in selecting contaminants of concern for bioaccumulation analysis by providing a general indication of the relative potential for various chemicals to accumulate in tissues.

The strategy for selecting contaminants of concern for the chemical analysis of tissue of organisms should include three criteria: (1) The target analyte is present at levels of potential concern in the sediment as determined by sediment chemical analyses. (2) The target analyte has a high potential to accumulate and persist in tissues. (3) The target analyte is of toxicological concern.

Analytes that might have a lower potential to bioaccumulate, but which are present at very high concentrations in the sediments, should also be included in the target list because the bioavailability of the compound might increase as organisms encounter high levels in sediments. In addition, compounds of a high accumulation potential and of high toxicological concern should be considered, even if present at low concentrations in the sediment.

Nonpriority-pollutant chemical components that are found in measurable concentrations in the sediments should be included as targets for the tissue analysis if review of the literature indicates that these analytes have the potential to bioaccumulate in animals (i.e., have a high

*K*ow or BCF) and persist in animal tissues, and are of toxicological concern.

9.5.2 Selection of Analytical Techniques (Biota)

At present, formally approved standard methods for the analysis of priority pollutants in tissues are not available. However, several studies conducted for EPA and other agencies have developed analytical methods capable of identifying and quantifying most organic and inorganic priority pollutants in tissues. The amount of tissue required for analysis is somewhat dependent on the analytical procedure. As a general guideline, 25 g (wet weight) of tissue should be delivered to the laboratory for organic analysis and 10 g delivered for metals analysis; an additional 25 g may be necessary for supplemental analyte determinations. The determination and recording of the moisture content of tissue samples is essential to convert data between wet-weight and dry-weight bases.

The detection limits achieved for target analytes in tissue depend on the sample size as well as the specific analytical procedure. The MDLs presented in a particular analytical method

should serve as goals for priority-pollutant tissue analysis. MDLs should be determined for all analytes according to guidance in 40 CFR 136 (Appendix A). Detection limits have to be specified based on the intended use of the data and specific needs of each evaluation.

The existing methods for the analysis of priority pollutants in tissue involve two separate procedures: one for organic compounds and another for metals. The recommended methods for the analysis of semivolatile organic pollutants are described in Extractable Toxic Organic Compounds, Standard Analytical Procedures of the NOAA National Analytical Facility (NOAA, 1989). These methods are currently being used in the NOAA National Status and Trends Program. The procedure involves serial extraction of homogenized tissue samples with methylene chloride, followed by alumina and gel-permeation column cleanup procedures that remove coextracted lipids. An automated gel-permeation procedure described by Krahn *et al.* (1988) is recommended for rapid, efficient, reproducible sample cleanup. The methylene chloride extract is concentrated and analyzed for semivolatile organic pollutants using GC with capillary fused-silica columns to achieve sufficient analyte resolution.

Chlorinated hydrocarbons (e.g., PCBs and chlorinated pesticides) should be analyzed by GC/ECD. It is recommended that PCBs be quantitated as specific congeners (Mullin *et al.*, 1984; Stalling *et al.*, 1987) and not by industrial formulations (e.g., aroclors) because the levels of PCBs in tissues result from complex processes, including selective accumulation and metabolism. See the discussion of PCB in Section 9.3.2. Lower detection limits and positive identification of PCBs and pesticides can be obtained by using chemical ionization mass spectrometry if necessary.

The same tissue extract is analyzed for other semivolatile pollutants (e.g., PAHs, phthalate esters, nitrosamines, phenols, etc.) using GC/MS as described by NOAA (1989), Battelle (1985), and Tetra Tech (1986b). These GC/MS methods are similar to EPA Method 8270 for solid wastes and soils (EPA, 1986). The lowest detection limits are achieved by operating the mass spectrometer in the SIM mode. Decisions to perform analysis of nonchlorinated hydrocarbons and the interpretation of resulting data should consider that many of these analytes are readily metabolized by most fish and many marine invertebrates.

If analysis of tissue samples for volatile priority pollutants is necessary, analytical methods are cited by Tetra Tech (1986b). The lipid content of the biological material is of importance in the interpretation of bioaccumulation information. A lipid determination should be performed on all biota submitted for organic analysis, and the method of Bligh and Dyer (1959) is recommended. If other methods are used, they should be referenced to results from Bligh and Dyer's method. If dioxin analysis is being performed, methods by Mehrle *et al.* (1988), Smith *et al.* (1984), or Kuehl *et al.* (1987) should be consulted.

The analysis for priority-pollutant metals involves a nitric acid or nitric acid/perchloric acid digestion of the tissue sample and subsequent analysis of the acid extract using AAS or inductively coupled plasma (ICP) techniques. Procedures for the digestion of tissue samples for priority-pollutant metals can be found in Tetra Tech (1986b). The methods used in the NOAA Status and Trends Program (NOAA, 1989) may also be used and are recommended when very low detection levels are required. Microwave technology may be used for tissue digestion to reduce contamination and to improve recovery of metals (Nakashima *et al.,* 1988). This methodology is consistent with tissue analyses performed for the NOAA Status and Trends Program, except for the microwave heating steps. Mercury analysis requires the use of cold- vapor AAS methods. The matrix interferences encountered in analysis of metals in tissue might require case-specific techniques for overcoming interference problems. If tributyltin analysis is being performed, the methods of Rice *et al.* (1987) or Uhler *et al.* (1989) should be consulted.

9.5.3 Quality Control

Section 14 presents a general discussion of appropriate QA/QC practices for tissue analysis. A procedural blank (to measure potential contamination from laboratory procedures) and a matrix spike (to measure the recoveries of the target analytes from a sample matrix) should be performed with each 10 - 20 samples. Triplicate analysis of one sample (to measure analytical precision) and appropriate SRMs (to measure analytical accuracy) should be performed with the same frequency as the blanks and matrix spikes. SRMs for organic priority pollutants in tissues are currently not available. The National Institute for Standards and Technology (NIST) is presently developing SRMs for organic analytes. Tissue matrix spikes of target analytes should be used to fulfill analytical accuracy requirements for organic analyses. SRMs for priority-pollutant metals include NRC dogfish liver tissue (DOLT-1), dogfish muscle tissue (DORM-1), and lobster hepatopancreas reference tissue (TORT-1); and IAEA fish flesh MA-A-2(TM) and mussel tissue MAM-2(TM). Marine reference materials and standards for inorganic constituents in tissue may be obtained from the organizations listed in Table 9-4.

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10.0 GUIDANCE FOR PERFORMING TIER II EVALUATIONS

10.1 TIER II: WATER-COLUMN EFFECTS

If a water-column limiting permissible concentration (LPC) determination cannot be made in Tier I, . 227.13 requires that the Tier II water-column evaluation be conducted to determine compliance with applicable marine water-quality criteria (WQC) (Section 5.1). "Bypassing" Tier II water-column testing is allowed only if there are no marine WQC for any of the contaminants of concern in the dredged material (Figure 3-2).

Tier II testing for WQC is a two-step process that uses one of three numerical models provided in Appendix B of this manual. The first step uses the model as a screen and assumes that all of the contaminants in the dredged material are released into the water column during the disposal process. The second step applies the same model, using the results from a chemical analysis of an elutriate prepared from the dredged material (Section 10.1.2.1).

10.1.1 Screen To Determine WQC Compliance

Step 1 of the Tier II water-column evaluation determines the need for additional testing by running the appropriate numerical model under the premise that all of the contaminants will dissolve into the water column. This is a conservative assumption and serves as a screen to reduce the evaluation effort for dredged material that will cause only minimal water-column impact. In a typical disposal operation, most contaminants remain associated with the dredged material that settles to the bottom and cause limited water-column impact during descent. Appendix B provides guidance on which numerical computer model should be applied to particular dredged-material disposal projects and the parameters that are necessary to run the programs. Versions of the models for use on IBM-compatible microcomputers and example applications are provided on the diskettes that can be found in the pocket inside the back cover of this manual.

The diskettes contain models appropriate to instantaneous discharges, continuous discharges, and hopper dredge discharges. The appropriate model for the proposed operation under consideration has to be selected according to the guidance in Appendix B. The output of the model is used to determine if additional testing is needed.

The model need be run only for the contaminant of concern that requires the greatest dilution. If the contaminant requiring the greatest dilution is shown to meet the LPC, all of the other contaminants that require less dilution will also meet the LPC. The contaminant that would require the greatest dilution is determined by calculating the dilution that would be required to meet the applicable marine WQC. To determine the dilution *D*, the following equation is solved for each contaminant of concern.

$D = (Cs\ Cwq) / (Cwq\ Cds)$

where

*C*s=concentration of the contaminant in the dredged material expressed as micrograms per liter (æg/L). [Note that most contaminant results are usually reported in micrograms per kilogram (æg/kg) dry weight. To convert the contaminant concentration reported on a dry-weight basis to the contaminant concentration in the dredged material, the dry-weight concentration must be multiplied by the mass of dredged-material solids per liter of dredged material];

*C*wq=applicable marine WQC in micrograms per liter (æg/L); and

*C*ds=background concentration of the constituent at the disposal site in micrograms per liter (æg/L).

Note that if the concentration of the constituent in the dredged material (*C*s) is less than the applicable marine WQC (*C*wq), no calculation is necessary since no dilution is required to meet the criteria. Note also that, if the ambient disposal-site water concentration (*C*ds) of a constituent is greater than the applicable WQC (*C*wq), water quality at the disposal site violates the marine WQC regardless of the proposed disposal operation, and the criteria cannot be met by dilution.

A data-analysis routine is available in the dispersion models (Appendix B) to perform the above calculations and identify the contaminant of concern that would require the greatest dilution.

The concentration of the contaminant that would require the greatest dilution is then modeled. The key parameters derived from the dispersion model are the maximum concentration of the contaminant in the water column outside the boundary of the disposal site during the 4-h initial-mixing period or anywhere in the marine environment after the 4-h initial-mixing period. If both of these concentrations are below the applicable marine WQC, the WQC LPC is met and no additional testing is required to determine compliance with the WQC. If either of these concentrations exceeds the WQC, additional testing is necessary, as described in Section 10.1.2. The procedure described above cannot be used to evaluate water-column impact; it can be used *only* to determine whether additional testing for potential water-column impact, as described in Section 10.1.2 and 11.1, is necessary.

10.1.2 Elutriate Analysis To Determine WQC Compliance

If the numerical mixing model applied in Section 10.1.1 shows that the WQC cannot be met if all of the contaminants in the dredged material dissolve into the water column during the disposal, an elutriate-chemical analysis must be conducted. Following an elutriate procedure with the dredged material and the subsequent chemical analysis, the model applied under Section 10.1.1 is run again with the new data that more closely estimates true disposal conditions. This second model run predicts whether or not the contaminant of concern that requires the greatest amount of dilution will meet or exceed

the LPC for WQC.

10.1.2.1 Dredged-Material Preparation (Standard Elutriate Test)

Prior to use, all labware should be thoroughly cleaned. Labware should be washed as appropriate for the analysis of the contaminants of concern. At a minimum, the labware should be washed with detergent, rinsed five times with tap water, placed in a clean 10% HCl acid bath for a minimum of 4 h, rinsed five times with tap water, and then thoroughly flushed with either distilled or deionized water.

The elutriate should be prepared by using water from the dredging site. If it is known at this time that there are no WQC for all of the contaminants of concern or that synergism is suspected, enough elutriate should be prepared for the chemical and for the water-column tests.

The elutriate is prepared by subsampling approximately 1 L of the dredged material from the well-mixed original sample. The dredged material and unfiltered water are then combined in a sediment-to-water ratio of 1:4 on a volume basis at room temperature (22ø ñ 2 øC). This is best accomplished by volumetric displacement. After the correct ratio is achieved, the mixture is stirred vigorously for 30 min with a magnetic stirrer. At 10-min intervals, the mixture is also stirred manually to ensure complete mixing. After the 30-min mixing period, the mixture is allowed to settle for 1 h. The supernatant is then siphoned off and centrifuged or filtered through a 0.45-æm-mesh filter to remove particulates prior to chemical analysis. If the elutriate is to be used for toxicity testing, refer to the procedures in Section 11.1.4.

10.1.2.2 Chemical Analysis

Analytical procedures for specific constituents in water are presented in Section 9.4.2.

10.1.2.3 Determination of WQC Compliance (Standard Elutriate Test)

A final LPC determination for WQC compliance is made following the second run of the appropriate numerical mixing model with the data from the chemical analysis of the elutriate. As stated in Section 10.1.1, guidance on the appropriate model to select and run for this analysis is provided in Appendix B. Copies of the models are also provided on the diskettes that can be found in the pocket inside the back cover of this manual.

Also as in Section 10.1.1, the model need be run only for the contaminant that requires the greatest dilution to make an LPC determination. This contaminant may or may not be the same as that run in the model under Section 10.1.1. Calculations must therefore be conducted for all of the contaminants detected during analysis of the elutriate to determine which one requires the greatest dilution. To determine the dilution D requirements, the following equation is solved for each contaminant of concern.$D = (Ce - Cwq) / (Cwq - Cds)$,

where

*C*e=concentration of the dissolved contaminant in the standard elutriate in micrograms per liter (æg/L);

*C*wq=applicable marine WQC in micrograms per liter (æg/L); and

*C*ds=background concentration of a constituent at the disposal site in micrograms per liter (æg/L).

Note that, if the concentration (*C*e) of the dissolved contaminants in the elutriate is less than the applicable marine WQC (*C*wq), no calculation is necessary since no dilution is required to meet the criteria. Note also that, if the ambient disposal-site water concentration (*C*ds) of a constituent is greater than the applicable WQC (*C*wq), water quality at the disposal site violates the marine WQC and the criteria cannot be met by dilution.

A data-analysis routine is available in the dispersion models to perform the above calculations and identify the contaminant of concern requiring the greatest dilution.

The concentration of the contaminant requiring the greatest dilution is then modeled. The key parameters derived from the model are the maximum concentration of the contaminant outside the boundary of the disposal site during the 4-h initial-mixing period and the maximum concentration anywhere in the marine environment after the 4-h initial-mixing period. These

values are compared with applicable marine WQC according to the guidance in Section 5.1.2, and a final LPC determination is reached for WQC compliance.

10.2 TIER II: THEORETICAL BIOACCUMULATION POTENTIAL (TBP)OF NONPOLAR ORGANIC CHEMICALS

The TBP is an approximation of the equilibrium concentration in tissues if the dredged material in question were the only source of contaminant to the organisms. The TBP calculation in Tier II is applied as a course screen to demonstrate LPC noncompliance of sediments that contain unacceptable concentrations of bioavailable contaminants of concern. At present the TBP calculation can be performed only for nonpolar organic chemicals (such as PCBs), although methods for making the calculation with metals and polar organic compounds are under development and may be added to this manual in the future. Therefore, a particular dredged material may contain contaminants of concern for which it is inappropriate to calculate TBP (e.g., polar organic compounds, organometals, and metals), and bioaccumulation evaluations of such dredged materials will require testing in Tier III or IV, as appropriate. However, even if the dredged material contains other contaminants of concern in addition to nonpolar organic contaminants of concern, it is still useful to calculate the TBP. The TBP provides an indication of the magnitude of bioaccumulation of nonpolar organic compounds that may be encountered in Tiers III and/or IV testing. Additionally, if the TBP of the nonpolar organic compounds meets the

decision guidance, the calculation may eliminate the need for further evaluation of these compounds and thereby reduce efforts in Tiers III and/or IV.

For the purposes of Tier II, nonpolar organic chemicals include all organic compounds that do not dissociate or form ions. This includes the chlorinated hydrocarbon pesticides; many other halogenated hydrocarbons; PCB, many PAHs including all the priority pollutant PAHs, dioxins, furans, etc. It does not include organic acids or salts, or organometallic complexes such as tributyltin or methyl mercury. Metals and metal compounds are not included.

The distribution in the environment of nonpolar organic chemicals is controlled largely by their solubility in various media. Therefore, in sediments they tend to occur primarily in association with organic matter (Karickhoff, 1981), and in organisms are found primarily in the body fats or lipids (Konemann and van Leeuwen, 1980; Geyer *et al.*, 1982; Mackay, 1982; Bierman, 1990). Therefore, bioaccumulation of nonpolar organic compounds from dredged material can be estimated from the organic carbon content of the material, the lipid content of the organism, and the relative affinities of the chemical for sediment organic carbon and animal lipid content.

The calculation of the TBP assumes that various lipids in different organisms and organic carbon in different sediments are similar and have similar distributional properties. Other simplifying assumptions are that chemicals are freely exchanged between the sediments and tissues and that compounds behave conservatively. In reality, compound size and structure may influence accumulation, and portions of organic compounds present on suspended particulates may have kinetic or structural barriers to availability. Two important assumptions implicit in the TBP calculations are: (1) There is no metabolic degradation or biotransformation of the chemical. (2) The sediment-associated chemical is totally bioavailable to the organism. Calculations based on these assumptions yield an environmentally conservative TBP value for the dredged material if the dredged material in question is the only source of the contaminant for the organism.

It is possible to relate the concentration of a chemical in one phase of a two-phase system to the concentration in the second phase when the system is in equilibrium. In calculating the TBP, interest is focused on the equilibrium distribution of a chemical between the dredged material or reference sediment and the organism. By normalizing nonpolar organic chemical concentration data for lipid content in organisms and organic carbon in dredged material or reference sediment, it is possible to estimate the preference of a chemical for either phase. This approach is based on the work of Konemann and van Leeuwen (1980) and Karickhoff (1981). McFarland (1984) took the approach one step farther. He calculated the equilibrium concentration of nonpolar organic chemicals that the lipids of an organism could accumulate as a result of exposure to dredged material would be about 1.7 times the organic carbon-normalized concentration of the chemical in the dredged material. Concentrations are directly proportional to the lipid content of the organism and the contaminant content of the dredged material or reference sediment, and are inversely proportional to the organic carbon content of the dredged or reference material (Lake *et al.* 1987).

This means that the chemical concentration that could result in an organism's lipids [the lipid bioaccumulation potential (LBP)] would theoretically be 1.7 times the concentration of that chemical in the sediment organic carbon. Rubinstein *et al.* (1987) have shown, based on field studies, that a value of 4 for calculating LBP is appropriate, and this is the value that is used in this manual. LBP represents the potential contaminant concentration in lipid if the sediment is the only source of that contaminant to the organism. It is generally desirable to convert LBP to whole-body bioaccumulation potential for a particular organism of interest. This is done by multiplying LBP by that organism's lipid content, as determined by lipid analysis or from reported data. Therefore, theoretical bioaccumulation potential (TBP) can be calculated as$$TBP = 4 (Cs / \%TOC) \%L ,$$

where TBP is expressed on a whole-body wet-weight basis in the same units of concentration as *C*s, and

*C*s=concentration of nonpolar organic chemical in the dredged material or reference sediment (any units of concentration may be used);

%TOC =total organic carbon content of the dredged material or reference sediment expressed as a decimal fraction (i.e., 2% = 0.02); and

%L=organism lipid content expressed as a decimal fraction (i.e., 3% = 0.03) of whole-body wet weight.

This calculation is based on work by McFarland and Clarke (1987), who also developed the nomograph in Figure 10-1 by which TBP can be determined graphically. Using the nomograph, it is possible to quickly estimate the TBP for organisms of various lipid contents, provided that the contaminant concentration *C*s and organic carbon content %TOC of the dredged-material or reference sediment are known. Even though the nomograph does not provide as precise an answer as the equation, it is sufficient for Tier II applications. Because the TBP does not predict expected environmental concentrations but indicates the upper range, exact evaluation is not necessary. The procedure for using the nomograph is as follows.

Step 1. Determine the lipid content of an organism of interest, either from previously reported values or from laboratory analysis, and express the lipid content as percent of whole-body wet weight rather than as a decimal fraction.

Step 2. Locate the value on the righthand vertical axis that corresponds most closely to that lipid content.

Step 3. Follow the sloped line until it intersects the dredged-material or reference-sediment concentration *C*s. *C*s may be expressed in any units of concentration and be selected from any of four ranges: 0.1-1.0; 1-10; 10-100; or 100-1000.

Step 4. From that point, read across to the lefthand vertical axis and select the TBP value from the appropriate sediment organic carbon column expressed as percent of sediment dry weight.

Step 5. Multiply the TBP by the factor (0.1, 1, 10, 100) corresponding to the selected *C*s range. The TBP will then be in the same units of concentration as *C*s.

The lipid scale and the *C*s scale of the nomograph can be changed by orders of magnitude by adjusting the TBP scale in the same manner. For example, if the organism of interest is a mussel having 0.3% lipid content, one would simply follow the 3% lipid line and divide the appropriate resulting theoretical bioaccumulation value by 10. If the dredged-material or reference-sediment concentration *C*s of a contaminant lies above or below the *C*s ranges shown on the nomograph, the units of concentration can be changed (e.g., change 0.02 parts per million to 20 parts per billion). Interpolation between lipid lines or between organic carbon columns is straightforward because all relationships are proportional. For example, for dredged material or reference sediment with an organic carbon content of 3%, the TBP would be 1/3 the TBP at 1% carbon, 5/3 the TBP at 5% organic carbon, 10/3 the TBP value at 10% organic carbon, or 20/3 the TBP at 20% organic carbon. The following illustration of the use of the nomograph determines the TBP of total PCB by a fish of 6% lipid content exposed to a sediment containing 4 ppm PCB and 4.6% total organic carbon. Follow the 6% lipid line to a *C*s value of 4 and then read across to the 5% organic carbon column to obtain a TBP of about 19 x 1 or 19 ppm. Because the organic carbon content of the sediment is actually 4.6% rather than 5%, a more precise estimate can be made by multiplying 19 by 5/4.6 to obtain a TBP of 20.6 ppm. This would be evaluated under guidance in Section 5.2 to determine whether a decision could be reached or further testing was necessary.

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11.0 GUIDANCE FOR PERFORMING BIOLOGICAL-EFFECTS TESTS

Biological-effects tests with the dredged material may be necessary if the evaluations in Tiers I and II conclude that the dredged material contains contaminants that might result in an unacceptable adverse impact to the benthic environment and/or the water column. Bioassays with whole sediment are used to determine the effects on benthic (bottom-dwelling) organisms; bioassays with suspensions/solutions of dredged material are conducted to determine the effects on water-column organisms. Bioassays should be conducted only in the tiers appropriate to provide the information necessary and sufficient for decisions.

The objective of water-column bioassays (if they are necessary) is to determine the potential impact of dissolved and suspended contaminants on organisms in the water column, after considering initial mixing period. Test organisms should be representative of sensitive water-column organisms existing in the vicinity of the disposal site.

The objective of benthic bioassays is to determine the potential impact of whole sediment on benthic organisms at and beyond the boundaries of the disposal site. The organisms used in testing should be representative of sensitive infaunal or epifaunal organisms existing in the vicinity of the disposal site. Benthic bioassays are intended to determine the potential toxicity of a dredged material as distinct from its physical effects. In tests similar to those described here, some animals are known to be affected by differences in sediment textures or absence of sediments (DeWitt *et al.*, 1988; McFarland, 1981). It is important, therefore, that test organisms and control and reference sediments be selected to minimize the artifactual effects of differences in grain size. If the sediment texture varies considerably between the dredged material and the control or reference sediments, either organisms insensitive to grain-size effects should be used or the effects of grain size have to be determined and considered when designing benthic bioassays and evaluating the test results. The purpose of the test is not to measure physical effects but to measure contaminant effects.

11.1 TIER III: WATER-COLUMN BIOASSAYS

Tests to evaluate dredged-material impact on the water column involves exposing test organisms to an elutriate dilution series containing both dissolved and suspended components of the dredged material. The test organisms are added to the exposure chambers and exposed for a prescribed period (usually 96 h). Tests with zooplankton and larvae may be run for shorter periods. The surviving organisms are examined at specified intervals to determine if the test material is producing an effect. An introductory guide to

general toxicity testing is presented in part 8000 of Standard Methods for the Analysis of Water and Waste Water (APHA, 1989). Biological-testing aspects of the Standard Methods guidelines may be followed as long as they do not conflict with the guidelines in this manual.

11.1.1 Species Selection

Paragraph 227.27(c) of the regulations defines appropriate sensitive water-column marine organism to mean at least one species each representative of phytoplankton or zooplankton, crustacean or mollusc, and fish. It is recommended that the test organisms be fish, crustaceans, and zooplankton. The test species may be from healthy laboratory cultures or may be collected from the vicinity of the disposal site or in an area of similar water quality and substrate sedimentology, but not within the influence of former or active disposal sites or other discharges. Ideally, the test species should be the same or closely related to those species that naturally dominate biological assemblages in the vicinity of the disposal site. Species characteristics to consider when designing water-column tests are

- Comply with paragraph 227.27(c)
- Are readily available year-round
- Tolerate handling and laboratory conditions
- Give consistent, reproducible response to toxicants
- Have related phylogenetically and/or by ecological requirements to species characteristic of the water column of the disposal site area in the season of the proposed disposal
- Can be readily tested as juveniles or larvae to increase sensitivity
- Are important ecologically, economically, and/or recreationally.

Note that the above test-species characteristics are not presented in order of importance, except that the first characteristic is mandatory.

With reasonable care, test organisms can be collected from wild populations and maintained in the laboratory with low mortality under controlled conditions. If the test species has not been used previously, a preliminary study should be conducted to assess the ability of the field-collected species to acclimate to laboratory conditions.

In addition to species occurring at the disposal site, other representative commercially available species or sensitive life stages of economically important species may be used. Mysids of the genera *Mysidopsis*, *Neomysis,* or *Holmesimysis* are highly recommended as test species. Embryo-larval stages of crustaceans, molluscs, or fish are also appropriate sensitive marine organisms. Adult fish and molluscs and large crustaceans are not recommended for water-column testing because of their generally greater resistance to contaminants. Appropriate test species are listed in Table 11-1.

Regardless of their source, test organisms should be collected and handled as gently as possible. Field-collected animals should be transported to the laboratory in seawater of

the same salinity and temperature as the water from which they were obtained. The animals should be held in the laboratory no longer than necessary, definitely no more than 2 weeks, before they are used. During this period, they have to be gradually acclimated to the salinity and temperature at which the test will be conducted. Animals from established laboratory cultures can be held indefinitely but may also need to be gradually acclimated to the test temperature and salinity if test conditions differ from holding conditions.

11.1.2 Apparatus

Water-column bioassays generally are run as static exposures for a period of 96 h. The exposures should be conducted in glass chambers equipped with covers to minimize evaporation. The size of the chambers depends on the size of the test species. All glassware has to be extremely clean. Before use, glassware should be washed with detergent, rinsed five times with tap water, placed in a clean 10% HCl acid bath for a minimum of 4 h, rinsed five times with tap water, and then thoroughly flushed with either distilled or deionized water.

Equipment and facilities have to be available to provide acceptable lighting requirements and temperature control. An environmental incubator or a water-bath system that allows temperature control within ñ1øC is recommended. A waterproof lightbox or light table is recommended for observing zooplankton and larvae.

11.1.3 Experimental Conditions

Water-column bioassays should be conducted under conditions known to be nonstressful to the test organisms. Salinity should be stable within +2o/oo and temperature within ñ2øC throughout the exposure period. Dissolved-oxygen concentration should not be allowed to fall below 40% saturation. The temperature, salinity, dissolved oxygen, ammonia, and pH in the test containers should be measured and recorded daily.

11.1.4 Experimental Procedures

Elutriate Preparation

Prior to use, all glassware should be thoroughly cleaned. Glassware should be washed with detergent, rinsed five times with tap water, placed in a clean 10% HCl acid bath for a minimum of 4 h, rinsed five times with tap water, and then thoroughly flushed with either distilled or deionized water. The elutriate should be prepared using water collected from the dredging site. Disposal-site water, clean seawater, or artificial sea/salt mixtures should be used as dilution water for the tests. If sea/salt mixtures are used for preparing the dilutions, the mixtures must be prepared in strict accordance with the manufacturer's instructions and allowed to age for a minimum of 1 week (with aeration) before use in any test. The elutriate is prepared by subsampling approximately 1 L of the homogenized dredged-material sample. The dredged material and unfiltered dredging-site water are then combined in a sediment-to-water ratio of 1:4 on a volume basis at room temperature

(22ø ñ 2øC). This is best accomplished by volumetric displacement. After the correct ratio is achieved, the mixture is stirred vigorously for 30 min with a magnetic stirrer. At 10-min intervals, the mixture is also stirred manually to ensure complete mixing. After the 30-min mixing period, the mixture is allowed to settle for 1 h. The liquid plus the material remaining in suspension after the settling period represents the 100% liquid plus suspended particulate phase. The supernatant is then carefully siphoned off, without disturbing the settled material, and immediately used for testing. With some very fine-grained dredged materials, it may be necessary to centrifuge the supernatant until the suspension is clear enough at the first observation time for the organisms to be visible in the testing chamber.

Test Design

The number of replicate exposure chambers per treatment and the number of organisms per exposure chamber should be determined according to the guidance in Section 13.1. A minimum of five replicates per treatment and 10 organisms per replicate is recommended unless Section 13.1 indicates otherwise. In all cases, the single most important concern is that the organisms not be stressed by overcrowding.

At least three concentrations of the dredged-material elutriate should be tested; recommended treatments are 100%, 50%, and 10% of the dredged-material elutriate. Water of the type in which the animals were held prior to testing should be included as control treatments. The toxicity of the dilution water should also be determined by conducting 100% dilution-water treatments to properly evaluate the test results.

The test organisms should be approximately of equal size and assigned randomly to the different treatments. Zooplankton and larvae are usually transferred with the aid of a pipette (Dinnel *et al.*, 1982). Care must be exercised so that air is not trapped on or under the animals during the transfer process. Larger animals may be transferred in fine-mesh nets. Animals that are dropped, physically abused, or exhibit abnormal behavior should be discarded.

The test chambers should be covered and placed in an incubator or water bath. The placement of the test containers in the incubator or water bath should be random. During the exposure period, the test medium should not be replaced, aeration should not be supplied (unless necessary to keep dissolved-oxygen concentration above 40% saturation), and the test solutions should not be stirred. Some species of crustaceans, particularly larval forms, will require feeding during the test. All food used must be analyzed to ensure that it is free of contaminants.

Recommended test duration is 48 h for zooplankton and larvae and 96 h for other organisms. For bivalve larvae, the ASTM (1988) procedure should be used. At 0, 4, 24, and 48 h (and perhaps 72 and 96 h), a lightbox or dissecting microscope is used to record the number of live animals in each chamber. Care must be exercised to minimize the stress to the animal. Only the number of living organisms are counted, not the number of dead. An animal is judged dead if it does not move either after the water is gently swirled

or after a sensitive part of its body is gently touched with a probe. At each observation, a pipette or forceps is used to remove dead organisms, molted exoskeletons, and food debris.

11.1.5 Quality-Control Considerations

If mortality is greater than 10% (30% mortality/abnormality for zooplankton tests) in the control treatment or in the dilution-water treatment for a particular test species, the test should be rejected and the bioassay repeated. Unacceptably high control mortality indicates that the organisms are being affected by stresses other than contamination in the material being tested. These stresses may be due to injury or disease, unfavorable physical or chemical conditions in the test containers, improper handling or acclimation, or possibly unsuitable or contaminated water. Species selection and the potential effects of these and other variables should be carefully examined in an attempt to reduce unacceptably high mortality if the test is repeated.

Reference toxicant tests should be performed routinely on all groups of organisms used in dredged-material testing in order to determine their relative health and vigor. Many chemicals may be used satisfactorily as reference toxicants (Lee, 1980). Reference toxicant tests are performed in the absence of sediment. A geometric dilution series of five unreplicated concentrations is used. Nominal concentrations are usually sufficient for reference toxicant tests, but measured concentrations are preferred. The concentration range should be selected to give greater than 50% mortality in at least one concentration and less than 50% mortality in at least one concentration. An initial pilot test using a very wide range of concentrations may be necessary to determine the proper concentration range for reference toxicant tests. Test duration is 24 h. Ten organisms per exposure chamber are sufficient. Reference toxicant tests usually are conducted under static conditions. For each species, mortality is determined and the LC50 is calculated as described in Section 13.2.2.

When data for a particular reference toxicant have been generated on at least five groups of organisms of a species, two standard deviations above and below the mean are established as the bounds of acceptability. When the next group of organisms of this species is tested with this reference toxicant, if the LC50 is within the bounds of acceptability, the group of organisms may be used for dredged-material testing. If not, their response is atypical of the population, and that group of organisms should not be used for testing. The data from each reference toxicant test are added to the database, and the bounds of acceptability are recalculated after each test to continually improve the characterization of the typical response of the species. Reference toxicant tests should be conducted at least monthly on each species cultured inhouse, and should be performed on each lot of purchased or field-collected organisms. The basic concept and application of reference toxicant tests is discussed by Lee (1980).

General quality assurance (QA) considerations applicable to biological tests are discussed in Section 14.

11.1.6 Data Presentation and Analysis

Data Presentation

Present the data for each test species in separate tables that include the following information.

- The scientific name of the test species
- The number of animals in each treatment at the start of the test
- The number of animals alive at each observation period
- The number of animals recovered alive from each chamber at the end of the test
- Additional information such as behavioral abnormalities.

Data Analysis

It is possible that no mortality will be observed in any of the treatments or that survival in the dredged-material treatments will be equal to or higher than in the control- or in the dilution-water treatments. In either of these situations, there is no need for statistical analysis and no indication of adverse effects attributable to the dredged material. If survival in the control- or dilution-water treatments is greater than the 100% dredged-material elutriate treatment, the data have to be evaluated statistically to determine whether the dredged-material suspension is significantly more toxic than either the control or the dilution water. If greater than 50% mortality occurs in any of the elutriate treatments, it might be possible to calculate an LC50 value (lethal concentration to 50% of the organisms in a sample). If less than 50% mortality occurs in any of the elutriate treatments, it is not possible to calculate an LC50. In such cases, the LC50 used in the model to determine compliance should be the 100% elutriate treatment. If the conditions are highly toxic, such that the 10% elutriate treatment has greater than 50% mortality, further dilution must be made (new treatments of less than 10% dredged-material elutriate) to attain a survival of greater than 50% and determine the LC50 by interpolation. Statistical procedures recommended for analyzing the test data are described in detail in Sections 13.2.1 and 13.2.2.

11.1.7 Determination of Compliance

The Tier III water-column effects evaluation involves running a numerical model to determine compliance with the LPC. A description of the models is given in Appendix B, and the models are provided on the diskettes that can be found in the pocket inside the back cover of this manual.

The diskettes contain models appropriate to instantaneous discharges, continuous discharges, and hopper-dredge discharges, as described in Appendix B. The appropriate model for the proposed operation under consideration has to be selected according to the guidance in Appendix B. Within that model, the Tier III water-column bioassay application is selected. The key parameters derived from the model for evaluating water-column toxicity in Tier III are the maximum concentration of dredged material in

the water column outside the boundary of the disposal site during the 4-h initial-mixing period, and the maximum concentration in the water column anywhere in the marine environment after the 4-h initial-mixing period.

The modeled concentrations of the dredged material (expressed as percentages) are compared to the LPC, as determined by 0.01 of the 48- or 96-h LC50, depending on the test duration. Both the maximum concentration outside the disposal-site boundary during the first 4 h and the maximum concentration at any point in the marine environment after 4 h are compared to 0.01 LC50. If both the modeled concentrations are less than 0.01 LC50, the discharge meets the LPC. If either of the modeled concentrations exceeds 0.01 LC50, the discharge does not meet the LPC.

11.2 WHOLE-SEDIMENT BIOASSAYS

Bioassays with whole sediment are designed to determine whether the dredged material is likely to produce unacceptable adverse effects on appropriate sensitive marine organisms. In acute tests, the test animals are exposed to the test sediment for 10 days and the number of survivors is recorded. For bioaccumulation tests, the concentration of contaminants is analyzed in test-organism tissue. In bioaccumulation tests, organisms are exposure to the dredged material for either 10 days or 28 days, depending on the contaminants of concern. The organisms used in both types of tests must represent the three categories of species specified in the regulations.

11.2.1 Species Selection

Appropriately sensitive benthic marine organisms are used to evaluate the potential benthic impact of dredged-material disposal. The regulations require that benthic bioassays be conducted with filter-feeding, deposit-feeding, and burrowing species [paragraph 227.27(d)]. Bioassay research on contaminated sediments (e.g., Word *et al.*, 1989; Gentile *et al.*, 1988; Rogerson *et al.*, 1985) and regulatory program experience since 1977 under the Marine Protection, Research, and Sanctuaries Act of 1972 (MPRSA) has shown that different species have various degrees of sensitivity to the physical and chemical composition of marine sediments.

To accurately evaluate potential benthic impact and regulatory compliance, the test species should be related as closely as possible, both phylogenetically and ecologically, to appropriate sensitive benthic marine organisms in the disposal-site area. Commercially important benthic species in the vicinity of the disposal site may also be considered for testing.

Consideration of species sensitivity is especially important because the sediment grain size is likely to vary substantially between the dredged material, the reference sediment, and the control sediment (DeWitt *et al.*, 1988; McFarland, 1981). If candidate test species are overly sensitive to the different grain sizes (i.e., excessive mortality in the control sediments) other, more grain-size tolerant species should be considered for the project.

A list of suitable bioassay species is presented in Table 11-2. However, it is strongly recommended that the selection of bioassays species for a particular dredged-material disposal project be made in consultation with regional regulatory and scientific personnel. Minimally, two different species that together cover the three species characteristics identified in paragraph 227.27(d) should be used to evaluate a disposal project. The following is a list of characteristics to consider for species selection for dredged-material evaluations.

- Comply with paragraph 227.27(c)
- Are readily available year-round
- Ingest sediments equally well
- Tolerate grain sizes of dredged material and control and reference sediments equally well
- Give consistent, reproducible response to toxicants
- Tolerate handling and laboratory conditions
- Are related phylogenetically and/or by ecological requirements to species characteristic of the benthic environment of the disposal site in the season of the proposed disposal
- Can be readily tested as juveniles or larvae to increase sensitivity
- Are important ecologically, economically, and/or recreationally.

Note that the above characteristics are not presented in order of importance, except that the first characteristic is mandatory.

Infaunal amphipods are strongly recommended as appropriate bioassay species for acute toxicity bioassays. Infaunal amphipods are

- Sensitive to benthic impact
- Readily available
- Tolerant of a wide range of grain sizes and laboratory exposure conditions
- Ecologically relevant to most dredged-material disposal sites
- In fulfillment of the three characteristics in paragraph 227.27(d).

Overall, infaunal amphipods are excellent bioassay organisms for short-term toxicity tests with whole sediment (Swartz *et al.,* 1979; Mearns and Word, 1982; Rogerson *et al.,* 1985; Gentile *et al.,* 1988; Word *et al.,* 1989).

Some polychaete species and juvenile forms of molluscs and crustaceans are also recommended as suitable bioassay organisms. Juvenile forms are especially useful because they are generally more sensitive than the adult forms and have direct ecological relevance. The identity of all species should be verified by experienced taxonomists, particularly for animals collected in the field. If the bioassay animals are also to be used in estimating bioaccumulation potential, the factors discussed in Section 11.1.1 for species selection should also be considered.

11.2.1.1 Infaunal Amphipods

As discussed above, infaunal amphipods are strongly recommended for conducting acute benthic bioassays. The information in Sections 11.2.1.2 through 11.2.1.5 is primarily for conducting amphipod bioassays. However, much of the information can also be used for testing other organisms.

11.2.1.2 Amphipod Handling

The number of test animals of each species in each replicate exposure chamber should be determined according to the guidance in Section 13.1. A minimum of 20 animals is recommended unless Section 13.1 indicates that fewer are sufficient. In all bioassays, the single most important concern is that the organisms not be stressed by overcrowding.

During collection, the animals should be handled as gently as possible, and placed in buckets containing about 3 cm of sediment and several liters of seawater. The animals should be transported to the laboratory in well-aerated water from the collection site. Benthic animals should be held in the laboratory in aquaria with a 5-cm layer of control sediment. This sediment should be sieved and contain no organisms that would adversely affect test results. Animals from established laboratory cultures can be held indefinitely. Animals collected from the field should be held no longer than necessary before they are used in testing. Infaunal amphipods should be held for no longer than 10 days. During the holding period, the organisms can be gradually acclimated, if necessary, to the temperature and salinity at which the toxicity test will be conducted.

11.2.1.3 Laboratory Apparatus for Amphipod Tests

The test system described by Swartz *et al.* (1985) for the phoxocephalid amphipod *Rhepoxynius abronius* is recommended for bioassays with this and other amphipod species. Some amphipods do not survive well under static conditions and, therefore, should be tested using only a continuous-flow or static-renewal test design. When static tests are not appropriate (i.e., if ammonia toxicity is suspected), a continuous-flow test system, similar to the systems described by Scott and Redmond (1989) and Word *et al.* (1989), is recommended. The American Society for Testing and Materials (ASTM Headquarters, 1916 Race St., Philadelphia, PA 19013) is preparing standardized guidance on conducting sediment bioassays with amphipods. The guidance will consist of a generic test design and species-specific appendices. When released by ASTM, this guidance for testing all species of amphipod may be followed on all points that do not conflict with this manual.

Larger aquaria (ò20 L) are recommended for larger species. Tests with large aquaria should be run under continuous-flow conditions with 90% of the water volume replaced at least once every 4 h. If a continuous-flow seawater supply is not available, the animals may be tested by using a static-renewal design. Seventy-five percent of the water in each exposure chamber should be renewed 1 h before and 48 h after test initiation and at 48-h intervals thereafter. Care should be taken to minimize resuspension of the sediments

during water changes. The water should be changed more frequently if acceptable water quality cannot be maintained.

All glassware has to be extremely clean. Before use, glassware should be washed with detergent, rinsed five times with tap water, placed in a clean 10% HCl acid bath for a minimum of 4 h, rinsed five times with tap water, and then thoroughly flushed with either distilled or deionized water.

The dilution water used in both flowthrough and static renewal tests should be of a temperature, salinity, and dissolved-oxygen concentration known to be nonstressful to the test organisms, and should be stable throughout the exposure period. The seawater should be filtered (20 æm), and the flow to the exposure chamber should be directed to achieve good mixing without disturbing the sediment on the bottom of the chamber. Static-renewal tests should be conducted in a water bath or environmental chamber to maintain the temperature within ñ1øC of the test temperature.

The procedures for collecting sediments (and animals and water if appropriate) are described in Section 8. The sediment samples should be stored as indicated in Table 8-1. The bioassay should include a control-sediment treatment, one or more reference-sediment treatments, and the dredged-material sample treatments.

Bioassays should be initiated as soon as practical after sediment collection, preferably within 2 weeks. However, if necessary, the sediment samples may be held up to 6 weeks before initiating bioassay tests. The number of replicate exposure chambers for the dredged material, reference, and control should be determined according to the guidance in Section 13.1. A minimum of five replicates is recommended, unless Section 13.1 indicates otherwise.

The quantity of sediment needed for the benthic tests depends on the size of the exposure chambers to be used. The test is conducted with either dredged material, reference sediment, or control sediment on the bottom of each exposure chamber. The sediment should be deep enough to meet the biological needs of the test organisms, i.e., allow organisms to burrow in their normal position, etc. In any case, it should be at least 2 cm deep.

11.2.1.4 Experimental Conditions for Amphipod Tests

Benthic bioassays should be conducted under conditions known to be nonstressful to the test organisms. Salinity should be appropriate for the geographic region and the test species and stable within ñ2o/oo and temperature within ñ2øC throughout the exposure period. Dissolved oxygen should be maintained above 40% saturation by gentle aeration if necessary, being careful not to resuspend the sediment. Water collected from the disposal site, clean seawater, or artificial sea-salt mixtures may be used to conduct the tests. If artificial sea-salt mixtures are used, they must be prepared in strict accordance with the manufacturer's instructions and allowed to age for at least 1 week (with aeration) before use in any tests. The standard test duration for acute toxicity bioassays on benthic organisms in Tier III is 10 days.

11.2.1.5 Experimental Procedures for Amphipod Tests

Prior to use in bioassays, all sediments must be thoroughly homogenized. Very small amounts of clean seawater may be added to facilitate mixing. If separation into liquid and solid phases occurs in posthomogenization storage, remixing will be required prior to using the sediment in the tests.

The reference and control sediments, as well as the dredged material being tested, may contain live organisms. Remove macrobenthic organisms by press-sieving the sediments through a 1-mm-mesh screen. The material remaining on the screen should be noted and discarded. Return the sieved dredged material to its storage container and hold it at 4øC. Use the sieved sediments as soon as practical after the macroinvertebrates are removed.

The experimental procedure described in Swartz *et al.* (1985) should be followed for preparing the exposure chambers for amphipod bioassays. For larger exposure chambers, the following procedure should be used. The control sediment, reference sediment, and the dredged material should be placed in their respective aquaria deep enough to meet the needs of the test organisms, but at least 2 cm deep on the bottom of the empty exposure chambers. The sediment on the bottom of the exposure chamber and any sediment suspended during placement in the exposure chamber should be allowed to settle for 24 h before introducing the test organisms. In continuous-flow tests, the flow should be established after most of the suspended sediment has settled, usually 12 to 24 h, but at least 1 h before introducing the test organisms. Water flow and any aeration should be directed to minimize the resuspension of sediments in the exposure chambers.

The use of flowthrough exposure systems is preferred to minimize the chances that stressful artifacts of experimental procedures will affect the results; static-renewal systems may be acceptable. If static-renewal systems are used, 75% of the water in each exposure chamber should be renewed 1 h before and 48 h after test initiation and at 48-h intervals thereafter. When the water is changed, be very careful not to resuspend settled material or test organisms.

Animals that have been collected in the field and kept in holding tanks with sediment can be recaptured by gently siphoning the sediment through a 1.0-mm screen. Handle the animals as little as possible and with the utmost care. Do not use any animals that are dropped, physically abused during capture or transfer, or exhibit unusual behavior. Specific handling requirements for amphipods are described in Swartz *et al.* (1985).

Divide the test animals randomly among finger bowls, or other suitable intermediate containers, equal in number to the number of exposure chambers in the test. Randomly place 20 individuals of each species in each container with water of the same temperature and salinity and from the same source as the water being used in the test. After 30 min, remove any dead animals or animals exhibiting unusual behavior and replace them with healthy individuals. If obvious mortalities exceed 10% during this period, discontinue the test and begin a new one. Reexamine species selection, collection, and holding techniques in an effort to reduce the unacceptably high mortality in the new test.

During the exposure period, daily-observation records should be kept of obvious mortalities, emergence of infaunal organisms, formation of tubes or burrows, and any unusual behavior. Also daily records of water-quality parameters (e.g., dissolved oxygen, salinity, temperature, pH) should be maintained. In static-renewal systems, ammonia concentrations should be measured to evaluate potential ammonia toxicity. Water-quality parameters may be kept within acceptable bounds by increasing the flow rate or frequency of water changes. Gentle aeration may also be used to keep dissolved-oxygen concentration above the 40% saturation level.

After the exposure period, the sediment in the exposure chambers is siphoned through a 0.5-mm-mesh screen. The material retained on the screen is gently rinsed with seawater and inspected for animals. Animals that show any response to gentle probing of sensitive parts should be considered alive. Specimens not recovered at the end of the test have to be considered as dead. Only living animals are counted, because dead animals may have decomposed or been eaten. If animals from the benthic bioassay are to be used in estimating bioaccumulation potential, the surviving specimens are gently and rapidly counted and then treated as described in Section 12.

11.2.2 Quality-Control Considerations

If greater than 10% mean mortality occurs in the control for a whole-sediment bioassay, the test must be repeated. Unacceptably high control mortality indicates that the organisms are being affected by important stresses other than contamination in the material being tested, and the test has to be repeated. These stresses may be due to injury or disease, unfavorable physical or chemical conditions in the test containers, improper handling or acclimation, or possibly unsuitable sediment grain size. Species selection and the potential effects of these and other variables should be carefully reexamined in an attempt to reduce unacceptably high mortality when the test is repeated.

Reference-toxicant tests should be performed routinely on all groups of organisms used in dredged-material testing. Many chemicals may be used satisfactorily as reference toxicants (Lee, 1980). Reference-toxicant tests are performed in the absence of sediment, even for animals to be used in benthic bioassays. The idea is to use short-term response to a standardized exposure as an indication of the relative health of the organisms. Sediment is unnecessary in the short reference-toxicant tests and, if used, would sorb the toxicant and invalidate the reference-toxicant test. A geometric dilution series of five unreplicated concentrations is used. Nominal concentrations usually are sufficient for reference-toxicant tests, but measured concentrations are preferred. The concentration range should be selected to give greater than 50% mortality in at least one concentration and less than 50% mortality in at least one concentration. An initial pilot test using a very wide range of concentrations may be necessary to determine the proper concentration range for the reference-toxicant tests. Test duration is 24 h. Ten organisms per exposure chamber are sufficient. Reference-toxicant tests are usually conducted under static conditions. For each species, mortality is determined and the LC50 is calculated as described in Section 13.2.2.

When data for a particular reference toxicant have been generated on at least five groups of organisms of a species, two standard deviations above and below the mean are established as the bounds of acceptability. When the next group of organisms of this species is tested with this reference toxicant, if the LC50 is within the bounds of acceptability, the group of organisms may be used for dredged-material testing. If not, their response is atypical of the population, and that group of organisms should not be used for testing. The data from each reference-toxicant test are added to the database, and the bounds of acceptability are recalculated after each test in order to continuously improve the characterization of the typical response of the species. Reference-toxicant tests should be conducted at least monthly on each species cultured in-house, and should be performed on each lot of purchased or field-collected organisms. The basic concept and application of reference-toxicant tests is discussed by Lee (1980).

General quality-assurance (QA) guidance that is applicable to bioassays is presented in Section 14.

11.2.3 Data Analysis

Data Presentation

Present the data for each test species in separate tables that include the following information.

- The scientific name of the test species
- The number of animals in each treatment at the start of the test
- The percent of animals recovered alive from each chamber at the end of the test
- Information regarding emergence, burrowing, tube building, and behavioral abnormalities
- Water-quality data for each test chamber for each day.

Statistical Analysis

If greater than 10% mean mortality occurs in the control, the test must be repeated. It is possible that no mortality will be observed in any treatments or that the total survival in the dredged material will be equal to or higher than survival in the reference sediments. In either of these situations, there is no need for statistical analysis and no indication of adverse effects due to the dredged material. If survival in the reference sediment is higher than in the dredged-material treatments, by more than the allowable percentage for the test species (see Section 6.2), the data have to be analyzed statistically to determine whether there is a significant difference in survival between the reference material and any dredged-material sample. Statistical procedures recommended for analyzing benthic bioassay data are described in detail in Section 13.2.3.

11.2.4 Determination of Compliance

Guidance on the use of the results to reach a decision is provided in Section 6.2.

11.3 TIER IV: CHRONIC-EFFECTS EVALUATIONS

At present, there are no routine methods available for assessing the chronic effects (i.e., effects on growth or reproductive processes) of contaminated sediments on benthic marine or estuarine organisms. However, a number of laboratory tests are under development or could be approved for this purpose. When standardized chronic-effects tests are approved, they will be incorporated in Tier III.

Ideally, chronic-effects bioassays measure reproductive effects on a sensitive sediment-ingesting, infaunal animal. A number of species of polychaetes and amphipods and certain species of bivalve molluscs (e.g., *Macoma* sp., *Yoldia limatula*) can be used. The primary disadvantage of this approach is that most species of infaunal polychaetes, amphipods, and molluscs have relatively long life cycles, and a test of several months or longer would be needed to accurately assess reproductive effects. It might be possible, however, to measure effects on growth the correlate with reproductive effects within a shorter exposure period. It might also be possible to measure bioenergetic alterations that correlate with reproductive suppression without conducting a full life-cycle test, as has been demonstrated with mysids (Carr *et al*., 1985).

11.4 TIER IV: CASE-SPECIFIC EVALUATIONS

Biological effects tests in Tier IV should be used only in situations that warrant special investigative procedures. In such cases, test procedures have to be tailored for specific situations, and general guidance cannot be offered in the context of this manual. Such studies have to be selected, designed, and evaluated as the need arises, with the assistance of administrative and scientific expertise from headquarters of EPA and the USACE, and other sources if appropriate.

In some cases, the potential for chronic benthic impact may be determined from properly designed and conducted field studies. The use of field studies for predictive purposes is valid only where there is a true historical precedent for the proposed operation being evaluated. That is, field study can be used only for maintenance dredging where the quality of the sediment to be dredged can be shown not to have deteriorated or become more contaminated since the last dredging and disposal operation. In addition, the disposal has to be proposed for the site at which the dredged material in question has been previously disposed, or for a site with similar sediment type supporting a similar biological community. Under these conditions, field studies can provide very realistic predictions of effect because benthic animals have been exposed throughout their life cycles to the chemical, physical, and biological conditions prevailing at the disposal site. Although field assessments are frequently of limited usefulness because of the above constraints, when the constraints are met, field assessments can be valuable.

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12.0 GUIDANCE FOR PERFORMING BIOACCUMULATION TESTS

Bioaccumulation refers to the accumulation of contaminants in the tissues of organisms through any route, including respiration, ingestion, or direct contact with contaminated sediment or water. The regulations require that bioaccumulation be considered as part of the environmental evaluation of dredged material proposed for ocean dumping. This consideration involves predicting whether there will be a cause-and-effect relationship between an animal's presence in the area influenced by the dredged material and an environmentally important elevation of its tissue content or body burden of contaminants above that in similar animals not influenced by the disposal of the dredged material. That is, it has to be predicted whether an animal's exposure to the influence of the dredged material is likely to cause a meaningful elevation of contaminants in its body.

Many marine organisms are capable of metabolizing some types of organic compounds to varying degrees, and the ability of each species to metabolize the specific contaminant(s) of concern influences the tissue concentration of those chemicals. Organic contaminants such as polychlorinated biphenyls (PCB) and other synthetic compounds can accumulate to high levels in animal tissues because they are highly resistant to metabolic degradation. Many polynuclear aromatic hydrocarbons (PAH), on the other hand, are readily taken up by many organisms, but might not be found in high concentrations in tissue because some of the parent compounds are rapidly metabolized. The metabolites are not easily quantified by standard analytical methods, but in many cases are potent toxicants that can adversely affect the organisms in which they occur. Relatively low concentrations of organic chemicals in tissues may thus suggest either low bioavailability and therefore low bioaccumulation, or that bioaccumulation was followed by metabolization. Therefore, it is important to evaluate PAH bioaccumulation in species that have only limited ability to metabolize them. Bivalve molluscs are generally considered to satisfy this requirement. For purposes of regulation, analyses of PAH in dredged material and organisms exposed to it should focus on the PAH on the priority pollutant list. The rationale for this recommendation is provided by Clarke and Gibson (1987).

12.1 TIER III: DETERMINATION OF BIOAVAILABILITY

Bioavailability tests are designed to evaluate the potential of benthic organisms to bioaccumulate contaminants of concern from the proposed dredged material. The *Guidance Manual: Bedded Sediment Bioaccumulation Tests,* by Lee *et al.* (1989), discusses bioaccumulation methodology in detail and may be followed on any matter that does not conflict with this manual. Tier III bioavailability tests are based on analysis of tissues of organisms after 10 or 28 days of exposure. The 10-day exposure test is

appropriate when all contaminants of concern are metals, whereas 28-day exposure tests should be used when any contaminant of concern is organic or organometallic (i.e., not an element). As discussed in Section 6.3, even though concentrations of these contaminants may not be at the steady state after 10 or 28 days, these tests determine the potential for bioaccumulation and provide the information for decision-making in the Tier III bioaccumulation evaluation.

12.1.1 Species Selection and Apparatus

Bioaccumulation tests must be conducted with appropriate benthic marine organisms. Paragraph 227.27(d) of the regulations defines this to mean that filter-feeding, deposit-feeding, and burrowing species must be submitted to tests that evaluate the bioaccumulation potential of contaminants in the proposed dredged material. These categories of species are broad and overlapping. The present recommendation is that a burrowing polychaete and a deposit-feeding bivalve mollusc be tested. These two organisms satisfy the requirements specified in paragraph 227.27(d) and are relevant to evaluating contaminant bioavailability at disposal sites.

Many species can metabolize PAH, thus giving a misleading indication of bioaccumulation potential. Therefore, it is essential that bioaccumulation studies include one or more species with very low ability to metabolize PAH. Bivalve molluscs are widely accepted as meeting this requirement.

Species characteristics to consider when selecting organisms for bioaccumulation tests are as follows.

- Comply with paragraph 227.27(d)
- Readily available year-round
- Provide adequate biomass for analysis
- Ingest sediments
- Tolerate grain sizes of dredged material and control and reference sediments equally well
- Tolerate handling and laboratory conditions
- Related phylogenetically and/or by ecological requirements to species characteristic of the disposal-site area
- Important ecologically, economically, and/or recreationally
- Inefficient metabolizers of contaminants, particularly PAH

Note that the above test-species characteristics are not presented in order of importance, except that the first characteristic is mandatory.

Regional scientists and regulatory personnel can be consulted for additional guidance for bioaccumulation-species selection. Examples of appropriate species for bioaccumulation testing are presented in Table 12-1

A minimum of several grams of tissue has to be available to allow measurement of

chemical concentrations (Section 9.5.2). In samples that do not contain sufficient tissue, it will be impossible to quantify the amount of contaminant present. Because data in the form of "concentration below detection limits" are not quantitative, it is vital that tissue sufficient to allow definitive measurement of concentration be collected for each species.

The apparatus to be used are those described for benthic bioassays in Section 11.2. In addition, aquaria with clean, sediment-free water are necessary to hold the organisms during the period required to void their digestive tracts. If the biological needs of the organisms require the presence of sediment, clean sand should be used.

12.1.2 Experimental Conditions

The test conditions are similar to that described in Section 11.2 for whole-sediment bioassays. Control animals should be sampled and archived at both the beginning and the end of bioaccumulation tests. If discrepancies are found during the data analysis (Section 12.1.4), the archived samples can be analyzed to obtain more information on the test conditions and possibly resolve the problems. Animals should not be provided food or additional sediment during the test. Animals to be used to evaluate bioavailability are taken from the dredged-material samples after 10 or 28 days of exposure.

It is necessary to empty or remove the digestive tracts of the animals immediately after sampling. Sediment in the digestive tracts may contain inert constituents and the contaminants of concern in forms that do not become biologically available during passage through the digestive tract.

If the animals are large enough to make it practical, the best procedure is to excise the digestive tracts as soon as possible after sampling. However, test organisms are seldom large enough to allow this, and most organisms have to be allowed to excrete the material. Organisms are placed in separate aquaria in clean, sediment-free water to purge their digestive tracts. Some polychaetes will pass material through the digestive tract only if more material is ingested. These animals have to be purged in aquaria with clean sand. Animals are not fed during the purging period. Fecal material is siphoned from the aquaria twice during the 24-h purging period. To minimize the possibility of loss of contaminants from the tissues, purging for longer periods is not recommended. The shells or exoskeletons of molluscs or crustaceans are removed and not included in the analysis. These structures generally contain low levels of contaminants and would contribute weight but little contaminants to the analysis. This would give an artificially low indication of bioavailability.

12.1.3 Chemical Analysis

Contaminants of concern to be assessed for bioavailability are those identified in Sections 4.2 and 9.5.1. Analytical procedures for contaminants of concern in tissue are presented in Section 9.5.2.

12.1.4 Data Analysis

The data should be presented in a table that lists the tissue concentration of each contaminant of concern measured in the organisms exposed to the dredged material and reference sediment.

To evaluate the significance of dredged-material contaminant bioaccumulation, the contaminant concentration of the test-organism tissue is statistically compared to *FDA Action Levels for Poisonous and Deleterious Substances in Fish or Shellfish for Human Food* (Table

6-1). (Refer to Figures 3-3.) Depending on the outcome of this comparison, tissue concentrations may also be statistically compared with those tissues of animals exposed to the reference material (Section 13.3.1.2). In some cases, the tissue concentration in animals exposed to one or more of the dredged-material samples may be less than or equal to that in animals exposed to the reference sediment. This in no way reflects adversely on the quality of the evaluation, but simply gives no indication of bioaccumulation potential for the contaminant, species, and dredged-material sample in question.

The sample of animals taken at the initiation of the exposure can be useful in interpreting results. It can add perspective to the magnitude of uptake during the exposure period, and in some cases has shown that elevated body burdens were not due to the dredged material or reference sediment but were already present in the organisms at the start of the test.

12.1.5 Determination of Compliance

Guidance on the use of the results of the determination of bioavailability in relation to FDA levels and bioavailability from reference sediment to reach a decision in Tier III is presented in Section 6.3.

12.1.6 Quality-Control Considerations

Reference-toxicant tests should be performed routinely on all groups of organisms used in dredged-material bioaccumulation testing in order to determine their relative health and vigor. Many chemicals may be used satisfactorily as reference toxicants (Lee, 1980). Reference-toxicant tests are performed in the absence of sediment, even for animals to be used in benthic bioaccumulation testing. The idea is to use short-term response to a standardized exposure as an indication of the relative health of the organisms. Sediment is unnecessary in the short reference-toxicant tests and, if used, would sorb the toxicant and invalidate the reference-toxicant test. A geometric dilution series of five unreplicated concentrations is used. Nominal (rather than measured) concentrations are usually sufficient for reference-toxicant tests. The concentration range should be selected to give greater than 50% mortality in at least one concentration and less than 50% mortality in at least one concentration. An initial pilot test using a very wide range of concentrations may be necessary to determine the proper concentration range for the reference-toxicant tests. Test duration is 24 h. Ten organisms per exposure chamber are sufficient. Reference-toxicant tests are conducted usually under static conditions.

For each species, mortality is determined and the LC50 is calculated as described in Section 13.2.2.

When data for a particular reference toxicant have been generated on at least five groups of organisms of a species, two standard deviations above and below the mean are established as the bounds of acceptability. When the next group of organisms of this species is tested with this reference toxicant, if the LC50 is within the bounds of acceptability, the group of organisms may be used for dredged-material bioaccumulation testing. If not, their response is atypical of the population, and that group of organisms should not be used for testing. The data from each reference-toxicant test are added to the database and the bounds of acceptability are recalculated after each test in order to continuously improve the characterization of the typical response of the species. Reference-toxicant tests should be conducted at least monthly on each species cultured inhouse, and should be performed on each lot of purchased or field-collected organisms. The basic concept and application of reference-toxicant tests is discussed by Lee (1980).

General quality-assurance (QA) guidance applicable to bioaccumulation testing is presented in Section 14.

12.2 TIER IV: DETERMINATION OF STEADY-STATE BIOACCUMULATION

Bioaccumulation evaluation at Tier IV provides for determination, either by laboratory testing or by collection of field samples, of the steady-state concentrations of constituents in organisms exposed to the dredged material as compared with organisms exposed to the reference material. Steady-state concentrations determined in the laboratory or in the field are used in the same way to make Tier IV decisions according to the guidance in Section 7.2.

12.2.1 Laboratory Assessment of Steady-State Bioaccumulation

Tier IV laboratory bioaccumulation testing is based on the American Society for Testing and Materials (ASTM) standard practice for conducting bioconcentration tests with fishes and saltwater bivalve molluscs (ASTM, 1984). The Tier IV test is a 28-day exposure to deposited dredged material from which steady-state concentration of contaminants in organism tissues is calculated based on time-series sampling.

12.2.1.1 Species Selection and Apparatus

The necessary species and apparatus are those indicated in Section 12.1.1 for Tier III bioaccumulation testing.

12.2.1.2 Experimental Conditions

Experimental conditions are the same as those described in Section 12.1.2 for

determination of bioavailability. A series of tissue samples taken during the exposure period provides the basis for determining the rate of uptake and elimination of contaminants by the organism. From these rate data, the steady-state concentration of contaminants in the tissues can be calculated, even though the steady state might not have been reached during the actual exposure. Steady state is defined for the purposes of this test as the concentration of contaminant that would occur in tissue after the organisms were exposed to the dredged or reference material for a very long time under constant exposure conditions.

At the time when the animals are placed in the aquaria to begin the exposure phase, an initial time-0 sample of each species is collected for tissue analysis. Additional tissue samples are collected from each of the five replicate reference and dredged-material aquaria at intervals of 2, 4, 7, 10, 18, and 28 days after exposure begins. Calculation of steady state as described in Section 13.3.2 requires that the data describe the inflection in the uptake curve. This might not require analysis of the samples collected at the later time intervals given above. If logistically practical, it may be cost-effective to submit the Day 2, 4, 7, and 10 samples to the laboratory for analysis and continue the experiment to collect the Day 18 and 28 samples. If the data from the first sampling times clearly include the inflection of the uptake curve, analysis of the samples from later intervals may not be necessary.

12.2.1.3 Chemical Analysis

Contaminants of concern to be assessed for bioaccumulation are those identified in Sections 4.2 and 9.5.1. Analytical procedures for contaminants of concern in tissues are presented in Section 9.5.2. As described in Section 12.1.2, sediment has to be removed from the digestive tracts of the animals before they are preserved.

12.2.1.4 Data Analysis

Complete tissue concentration data for all tissue samples should be presented in a table. Recommended statistical methods for fitting a curve to the data to determine steady-state concentration in the tissue are presented in Section 13.3.2. The statistical procedures use an iterative curve-fitting process to determine the key variables ($k1C$s the uptake rate-constant times the contaminant concentration in the sediment, and $k2$ the depuration rate constant). An initial value for Cs has to be supplied. When the sediment concentration of the contaminant of concern is used, the ratio of $k1/k2$ is the sediment bioaccumulation factor (BAF) (Lake *et al.* 1987; Rubinstein *et al.*, 1987), the ratio of steady-state tissue concentration to sediment concentration.

12.2.1.5 Determination of Compliance

Decisions are based on the magnitude of bioaccumulation from the dredged material, and its comparison with the FDA levels, steady-state bioaccumulation from the reference sediment, and the body burden of reference organisms. Guidance for making decisions in Tier IV based on these comparisons is presented in Section 7.2.

12.2.1.6 Other Considerations

Although procedures for performing bioavailability and steady-state bioaccumulation tests have been discussed separately, it may be practical to combine these procedures in practice. This can be done by following the steady-state bioaccumulation procedure, but initially analyzing only the 10- or 28-day sample. If the use of the data from this analysis as part of the Tier III bioavailability evaluation does not provide for decision-making, then the remaining time-series samples may be analyzed and used in the Tier IV steady-state bioaccumulation evaluation.

12.2.1.7 Quality-Control Considerations

Guidance on quality-control (QC) considerations for bioaccumulation testing is provided in Section 12.1.6.

12.2.2 Field Assessment of Steady-State Bioaccumulation

Field-sampling programs overcome difficulties related to quantitatively considering field-exposure conditions in the interpretation of test results, since the animals are exposed to the conditions of mixing and sediment transport actually occurring at the disposal site in question. Difficulties related to the time required to conduct laboratory bioaccumulation studies are also overcome if organisms already living at the disposal site are used in the field bioaccumulation studies. The use of this approach for predictive purposes is technically valid only where there is a true historical precedent for the proposed operation being evaluated. That is, it can be used only in maintenance dredging where the quality of the sediment to be dredged can be shown not to have deteriorated or become more contaminated since the last dredging and disposal operation. In addition, the disposal has to be proposed for the site at which the dredged material in question has been previously disposed or for a site of similar sediment type supporting a similar biological community. Knowledge of the contaminant body burden of the organisms living around the proposed disposal site is used in evaluating bioaccumulation results in Tier IV (Section 7.2).

12.2.2.1 Apparatus

The following is a general description of the major items required for field assessment of bioaccumulation potential. Additional miscellaneous equipment will have to be furnished.

- A vessel capable of operating at the disposal site and equipped to handle benthic sampling devices.

  Navigation equipment has to be sufficient to allow precise positioning.
- Sampling devices such as a box corer, Smith-MacIntyre or other benthic grab. Corers are less satisfactory because they sample a smaller surface area and have a greater penetration than is needed.

- Stainless steel screens of 1-mm mesh to remove animals from the sediment.
- Tanks for transporting the animals to the laboratory in collection-site water.
- Laboratory facilities for holding the animals prior to analysis.
- Chemical and analytical facilities as required for the desired analyses.

12.2.2.2 Species Selection

The species selected for analysis have to be present in sufficient numbers for collection of an adequate sample at all stations. The same species have to be collected at all stations because bioaccumulation cannot be compared across species lines.

For each species at each station, a minimum of several grams of tissue has to be collected to allow measurement of chemical concentrations. In samples that do not contain sufficient tissue, it will be impossible to quantify the amount of contaminant present. Because data in the form of "concentration below detection limits" are not quantitative, it is vital that sufficient tissue to allow definitive measurement of concentration be collected for each species at each station. The ability to obtain sufficient tissue is a critical factor in selecting species for use in bioaccumulation studies, and in determining the practicality of the field assessment approach.

If possible, several samples of sufficient size for analysis should be collected at each sampling station to provide a statistical estimate of variability in tissue content of the contaminants of concern. Collection of more than one sample per station, however, may prove impractical if a composite of many small organisms have to be used or if suitable organisms are not abundant at the disposal site.

To minimize the numbers and collection effort required, it is desirable to select the largest appropriate species. However, highly mobile epifauna (such as crabs, lobsters, shrimp, and fish) should not be used, because a cause-and-effect relationship cannot be established between their location when collected and their body burden at the time of collection. Therefore, relatively immobile species that are fairly large, such as bivalves, some gastropods, large polychaetes, etc., are the most desirable organisms. Any relatively immobile species collectable in sufficient numbers at all stations may be used, but the required collection effort increases sharply as organism size decreases.

As discussed at the beginning of this Section, many species can metabolize PAH, thereby giving a misleading indication of bioaccumulation potential. Therefore, it is essential that bioaccumulation studies include one or more species with very low ability to metabolize PAH. Bivalve molluscs are widely accepted as meeting this requirement.

12.2.2.3 Sampling Design and Conduct

Sufficient tissue to obtain definitive body-burden values has to be collected from each of at least three stations within the disposal-site boundaries. It is mandatory that several stations be sampled, rather than collecting all of the animals at one station. This will provide a measure of the variability that exists in tissue concentrations in the animals in

the area. Samples from all stations should be collected on the same day if possible, and, in any case, within 4 days.

12.2.2.4 Basis for Evaluation of Bioaccumulation

Tier IV bioaccumulation, whether based on laboratory or field assessment, is evaluated (Section 7.2) by comparison to contaminant concentrations in field organisms living around, but not affected by, the disposal site. This is very similar to the reference-area approach (Section 3.1.2.1). To generate these data, at least three stations have to be located in an uncontaminated material sedimentologically similar to that within the disposal site, in a direction perpendicular to the net bottom transport. Data from these sites will provide the level of contaminants in tissues to which those levels found in organisms exposed to the dredged material may be compared. If the direction of net bottom transport is not known, at least six stations surrounding the disposal site should be established in sediments sedimentologically similar to those within the disposal site.

In all cases, it is mandatory that several stations be sampled, rather than collecting all of the animals at one station. This will provide a measure of the variability that exists in tissue concentrations in the animals in the area. Samples from all stations should be collected on the same day if possible, and, in any case, within 4 days.

12.2.2.5 Sample Collection and Handling

When the collection vessel has been positioned, make repeated collections at the same spot until an adequate tissue volume is obtained. Gently wash the sediment obtained by the sampler through 1-mm-mesh stainless-steel screens, and place the retained organisms of the desired species in holding tanks. Never retain an animal that shows any indication of injury.

Label the samples clearly and return the animals to the laboratory, being careful to keep them separated and to maintain nonstressful levels of temperature and dissolved oxygen. In the laboratory, maintain the samples in clean water in separate containers. Do not place any sediment in the containers and do not feed the animals. Immediately discard any organisms that die.

It is necessary to remove sediment from the digestive tracts of the animals because it may contain inert constituents and the contaminants of concern in forms that do not become biologically available during passage through the digestive tract. If the animals are large enough to make it practical, the best procedure is to excise the digestive tracts as soon as possible after collection. However, animals are seldom large enough to allow this, and most organisms have to be allowed to excrete the material. Surviving organisms are placed in separate aquaria in clean, sediment-free water to purge their digestive tracts. Some polychaetes will pass material through the digestive tract only if more material is ingested. These animals have to be purged in aquaria with clean sand. Animals are not fed during the purging period. Siphon fecal material from the aquaria twice during the 24-h purging period. Purging for longer periods of time is not recommended to minimize

the possibility of loss of contaminants from the tissues.

Also remove the shells or exoskeletons of molluscs or crustaceans. These structures generally contain low levels of contaminants and would contribute weight but few contaminants if they were included in the analysis. This would give an artificially low indication of bioaccumulation.

12.2.2.6 Chemical Analysis

The contaminants of concern to be assessed for bioaccumulation are those identified in Sections 4.2 and 9.5.1. Analytical procedures for specific constituents are presented in Section 9.5.2.

12.2.2.7 Data Analysis

Complete tissue concentration data for all samples should be presented in table format. Recommended statistical methods are presented in Section 13.3.

12.2.2.8 Determination of Compliance

Decisions are based on the magnitude of bioaccumulation in organisms collected within the boundaries of the disposal site, and its comparison with bioaccumulation in organisms living around the disposal site, but not affected by the site. Guidance for making regulatory decisions based on this comparison is presented in Section 7.2.

12.3 REFERENCES

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13.0 STATISTICAL METHODS

This Section presents the appropriate statistical methods for analyzing data from bioassays and bioaccumulation tests. The methodology is not intended to be exhaustive, nor is it intended to be a "cook-book" approach to data analysis. Statistical analyses are routine only under ideal experimental conditions. The methods presented here will usually be adequate for the tests conducted under the conditions specified in this document. An experienced applied statistician should be consulted whenever there are questions.

The following are examples of departures from ideal experimental conditions that may require additions to or modifications of the straightforward statistical methods presented in this chapter:

- Unequal numbers of experimental animals assigned to each treatment container, or loss of animals during the experiment
- Unequal numbers of treatment replications of the treatments (i.e., containers or aquaria)
- Measurements scheduled at selected time intervals actually performed at other times,
- Different conditions of salinity, pH, dissolved oxygen, temperature, etc., among exposure chambers
- Differences in placement conditions of the testing containers, or in the animals assigned to different treatments.

The following statistical methods will be presented as each applies to a specific test procedure.

- Sample-size determinations
- Data-scale transformations
- Variance homogeneity tests
- Two-sample *t*-tests
- Analysis of variance (ANOVA)
- Multiple comparisons among treatment means
- Confidence interval calculations

The statistical methods are illustrated in this manual with example IBM PC programs using the SAS System (SAS Institute, 1985). This manual does not constitute official endorsement or approval of these commercial hardware or software products. Other equally acceptable hardware and software products are commercially available and may be used to perform the necessary analyses. Whenever it is necessary to write original programs to perform statistical analysis, the appropriate- ness of the techniques and

accuracy of the calculations must be very carefully verified and documented.

13.1 SAMPLE-SIZE CONSIDERATION

The goal in analyzing the bioassay and bioaccumulation test data is to determine whether the mean effect of exposure to a dredged material is significantly greater than the mean effect of exposure to a reference sediment. For both the dredged material and the reference sediment, the data consist of responses measuring the effect of the material on *k* organisms in each of *n* replicate samples. In Sections 10 and 11, where guidance for performing the various tests is provided, *k* is usually set at 10 to 20 organisms per replicate, depending on the test. In the two-sample statistical test for significance, it is necessary to determine the number of replicate measurements per treatment group *n,* which must be taken to detect differences between the groups, while also taking cost and handling time into consideration.

Two formal hypotheses underlie the statistical analysis of data in the two sample situations. Let R denote the mean effect of exposure to the reference sediment R, and let D denote the mean effect of exposure to the dredged material D. Then, these two hypotheses are defined as follows.

Null hypothesis

H0: D = R .

There is no difference in mean effect between the treatment (dredged material) and reference groups of animals.

Alternative hypothesis

H1: D > R .

The mean effect of the dredged material is greater than the mean effect of the reference sediment.

Our test of hypothesis will either reject H0 for H1 or will fail to reject H0. A "one-tailed" test is used because there is little concern about identifying a lower exposure effect in dredged material than in reference sediment.

In performing the test of hypothesis, and in determining the sample size to use in the test, the evaluator must be aware of the probabilities for two types of errors that can occur in the conclusion. A Type I error occurs when, after analysis of the data, H0 is rejected when it was actually true. A Type II error occurs when H0 is not rejected when it actually should have been rejected. The probability of a Type I error is often represented by the letter ; the probability of a Type II error is often written as .

In the example, a Type I error occurs when it is concluded that the mean effect of the dredged material is greater than the mean effect of the reference sediment when, in fact, the true mean effect of the dredged material is no greater than that for the reference

sediment. On the other hand, a Type II error occurs when it is concluded that there is no difference in mean effects of the two materials when, in fact, the true mean effect of the dredged material is greater.

The power of a statistical test is defined as 1 , which is the probability of rejecting H0 when it should be rejected. In this example, the power is the probability of concluding that mean effect is greater in the dredged-material group when, in fact, this is true. The conclusions are based on performing a two-sample *t*-test. In this type of test, the power depends on the actual differ- ence in mean effects that we wish to detect, the (pooled) standard deviation of the responses within each treatment group, and the (common) sample size within each treatment group. Under ideal circumstances, the experimenter wishes to maximize the power subject to a fixed probability of Type I error.

More accurately, the power of a statistical test depends on ë/å, where ë is ³ D R³ and å is the pooled standard deviation of responses within the two treatment groups, as well as on the sample size. For a fixed sample size, large values of ë/å lead to high power. However, if ë/å is treated as fixed, the power can be increased by increasing the sample size. Thus, the experimenter will decide in advance what size difference in treatment means ë is necessary for the test to detect, relative to the variation å within treatment groups, and then choose sample size *n* large enough to achieve a given power.

If the response is highly variable within treatment groups, only large differences in the true mean effect between dredged material and reference groups are likely to be detected. Conversely, if the response is less variable, smaller differences in true mean effect between the dredged material and reference groups can be detected. This is due to the relationship between power and the ratio ë/å.

For a selected sample size, Table 13-1 presents the calculated power (in percent) for the one-tailed test (Cohen 1977), assuming a Type I error probability of 0.05 and ë/å = 1. Thus, it is assumed that the variability within treatment groups is equal to the difference in mean effects that are detected. From this table, it is seen that for a sample size of five per treatment group, the power is 0.43. This means that a difference in mean effect of one standard deviation between the dredged material and the reference sample would be detected 43% of the time. Similarly, to detect a true difference in mean effect of one standard deviation 80% of the time at = 0.05, the number of replicates per treatment would have to be approximately 13.

Throughout this document, a minimum of five replicate samples from the test containers is recommended for each treatment level. Experience has shown this number of replicates to be cost-effective and easy to manage. However, as shown, it is important to select a sample size large enough to achieve a high statistical power in detecting differences in the treatment groups.

13.2 BIOLOGICAL EFFECTS

13.2.1 Tier III Water-Column Bioassays

The objective of the analysis of Tier III water-column toxicity test data is to assess the evidence for reduced survival due to toxicity of suspended plus dissolved dredged-material constituents, and to calculate the median lethal concentration (LC50) of the material from the serial dilution experiment described in Section 11.1.4.

At the end of the exposure period, the effects, if any, on the survival of the test organisms should be clearly manifest in the 100% concentration (undiluted) test container. When the dilutions were prepared with other than control water, the dilution-water treatment is preferred over the control water for the following statistical analysis. The appropriate statistical test for detecting a significant difference in survival between two independant samples, i.e., the dilution water and the 100% concentration, is the two-sample *t*-test (Snedecor and Cochran, 1980). The usual *t* statistic for testing the equality of means 1 and 2 from two independent samples with *n*1 and *n*2 observations is

$$t = (\bar{x}_1 - \bar{x}_2) / \sqrt{s^2 (1/n_1 + 1/n_2)}$$

where *s*2, the pooled variance, is calculated as

$$s^2 = [(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2] \div (n_1 + n_2 - 2)$$

and where *s*21 and *s*22 are the sample variances of the two groups. This statistic is compared with the student-*t* distribution with *n*1 + *n*2 2 degrees of freedom.

The use of this *t* statistic depends on the assumption that the variances of the two groups are equivalent. Under the assumption of unequal variances, the *t* statistic is computed as

$$t = (\bar{x}_1 - \bar{x}_2) / \sqrt{s_1^2/n_1 + s_2^2/n_2}$$

This statistic is compared with the student-*t* distributions with degrees of freedom given by Satterthwaite's (1946) approximation:

$$df = \frac{(s_1^2/n_1 + s_2^2/n_2)^2}{(s_1^2/n_1)^2/(n_1 - 1) + (s_2^2/n_2)^2/(n_2 - 1)}$$

The assumption of equal variances can be tested by comparing the folded *F* statistic with the *F* distribution having *n*1 - 1 and *n*2 - 1 degrees of freedom. *F* is calculated as

$$F' = (\text{larger of } s_1^2, s_2^2) / (\text{smaller of } s_1^2, s_2^2)$$

When *F* is large, the hypothesis of equal variance is more likely to be rejected. This *F* test is a two-tailed *F* test since we do not specify which variance is expected to be larger.

Table 13-2 contains sample data from a 96-h water-column bioassay using a seawater control and dissolved plus suspended dredged-material constituents at four serial dilutions. In this example, mean mortality in the control is less than 10%, indicating the acceptability of the test.

Figure 13-1 illustrates an SAS/PC program that will perform a two-sample *t*-test between control and the 100% concentration, and a Levene's test of the homogeneity of sample variances. The results from this program are given in Figures 13-2 and 13-3. Figure 13-2 lists data (produced of by the PROC PRINT; statement) and the two-sample *t*-test results (produced by the statement PROC TTEST COCHRAN; and the next three statements). Three *t*-test results are given: two versions of the *t*-test for assuming unequal variances, and one for use if the variances in the two treatments are equal.

The *F* statistic is used in testing the hypothesis that the sample variances of the control data and 100% concentration data are equal (Steel and Torrie, 1980). The *F* test in the example in Figure 13-2 is significant at the 0.064 level, indicating that if the true variability of responses was equal between the two groups, then we expect to observe data with as much or more unequal variability as we had in this set of data only 6.4% of the time. Since this probability is so low, these data suggest that variances in the two groups are in fact not equal. The test is on the verge of being significant, if we are judging significance at the 0.05 level.

In such cases, it is usually prudent to use the *t*-test for unequal variances. Choosing this approach, the *t*-test, assuming unequal variances, indicates a significant difference (Prob>|*T*| = 0.0001) in survival between these two treatments. Significance probabilities for all of the *t*-tests in the SAS results are two-tailed probabilities. For this application, we are concerned about dredged-material samples with an effect greater than the control, and it is not important to detect dredged-material samples that have less effect than the control. To obtain the one-tailed or directional probabilities that we wish here, we divide the two-tailed probabilities by 2 and consider the sign of the *t* statistic. Here, we are comparing the response in the control to the response in the 100% concentration. In this case, the control mean is greater than the mean of the 100% concentration group and, therefore, the *t* statistic is positive. Considering the *t*-test for unequal variances, the results are significant ($p = 0.00005$) and in the direction that we consider important; i.e., there is statistically significant increased mortality in the 100% concentration.

The *F* test of equality of variances, given by the SAS program, is sensitive to departures from the assumption that these samples have been taken from populations with an underlying normal probability distribution. Figure 13-3 presents the results of a Levene's test, which is not sensitive to this assumption for reasonable samples sizes. This test is based on an ANOVA of the absolute deviations of the responses from the response group mean. Larger sample variances indicate larger absolute deviations. Results of Levene's test show that there is weaker evidence (Pr > *F* = 0.093) than in the *F* test that we should reject the hypothesis of equal variances. That is, if there really were no difference in variances, then the probability of obtaining an *F* value as large as or larger than the one obtained from these data is almost 10%. In this example, the *t*-test shows that there is a

statistically significant difference between control and 100% concentration groups in the mean number of surviving organisms, whether or not equal variances are assumed for the two groups.

13.2.2 Calculating Median Lethal Concentration

In the Tier III water-column bioassays it is recommended (Section 11.1.5) that the median lethal concentration (LC50) be calculated for each observation time of the experiment. Confidence intervals on these values are used to assess whether the toxicity of the dredged material exceeds the limiting permissible concentration (LPC). It is not possible to calculate every LC50 unless at least 50% of the test organisms die in at least one of the serial dilutions. Experience indicates that

often this does not occur for earlier time periods. If it is not possible to calculate an LC50, then the LC50 is assumed to be 100%.

LC50 calculations are recommended also for reference toxicant tests to determine the relative health of the organisms used in bioassay and bioaccumulation testing.

Table 13-2 gives examples of data from a 96-h water-column bioassay. We see from these data that intermediate concentrations of the dredged material show intermediate proportions of surviving test organisms. The aim, therefore, is to apply some statistical method to these data to estimate the LC50 concentration at which 50% of the animals in the population would die. Calculating a 95% confidence interval using the sample LC50 signifies that there is only a 5% probability that the interval contains the true LC50 of the population of test organisms.

Because opinions vary about the most appropriate statistical method for calculating the LC50, this implementation manual recommends using two or more of the procedures in the following citations to calculate the LC50. Stephan (1977) and Gelber *et al.* (1985) provide careful reviews of LC50 estimation procedures. In addition, EPA (1985) discusses in detail the mechanics of calculating the LC50 by using current methods and contains, as an appendix, computer programs for each statistical method.

Compliance with the regulations is determined according to the Tier III guidance in Section 6.1.

13.2.3 Tier III Benthic Bioassays

The objective of a statistical analysis of Tier III benthic-bioassay data is to determine the strength of the evidence for concluding that the dredged-material samples are significantly more toxic to marine benthic infauna than are the reference-sediment samples. The test procedure is described in Section 11.2.

This objective can be accomplished by using an analysis of variance (ANOVA) procedure and an associated multiple comparison procedure known as Dunnett's test. These statistical techniques are discussed by Snedecor and Cochran (1980), Steele and

Torrie (1980), SAS Institute (1985), and Dunnett (1964).

Table 13-3 presents survival data from a hypothetical benthic bioassay. In this example, mean mortality in the control is less than 10%, indicating the acceptability of the test. The ANOVA procedure assumes that the survival responses are independently and normally distributed with a common variance among treatment levels. For instance, if *Xij* is the survival response (such as number of survivors) for the *i*th treatment level and *j*th replicate, then we assume that the underlying distribution of *Xij* is normal with mean æi and variance å2.

In other words, the treatment levels can have different means, but all levels have the same variance. The assumptions of normality and constant variance are not always met. AlthoughANOVA is fairly robust to deviations from these assumptions when sample sizes are equal, a test of the validity of these assumptions is recommended before performing the ANOVA. Bartlett's test (Snedecor and Cochran, 1980), the *F* test (Section 13.2.1), or Levene's test (Section 13.2.1) may be used to test for homogeneity of variances. If the raw data do not satisfy these assumptions, a mathematical transformation can sometimes be applied to the data, which will confer a more normal distribution to the transformed data and will stabilize the variance among treatment levels (Natrella, 1963). For example, a common transformation for proportions (such as percent survival) is

$$Y_{ij} = \text{arcsine}\sqrt{p_{ij}}$$

where *pij* is the proportion of survivors at the *i*th treatment level and for the *j*th replicate, i.e., *pij* = X*ij*/k. We recommend that the survival proportion be used as the treatment response for analysis. If the data do not satisfy the ANOVA assumptions of normality and constant variance, we recommend that the arcsine/square-root transformation presented above be used prior to performing the ANOVA, although any transformation that increases normality and stabilizes variance among treatments may be used.

Another common transformation used to stabilize the variance is the logarithmic transformation. It is used when the standard deviation increases in direct proportion to the mean, i.e., when those treatments with larger means also have larger standard deviations. The transformation is simply

$$Y_{ij} = \log(X_{ij})$$

Either natural or base-10 logarithms are commonly used.

Figure 13-4 illustrates an SAS/PC program that performs an ANOVA on the transformed survival proportions calculated from Table 13-3. In addition to the ANOVA, this program includes an analysis of the total number of survivors using a nonparametric Kruskal-Wallis test (Daniel, 1978) for comparison. The nonparametric test often is performed when the distributional assumptions of the parametric ANOVA test cannot be verified. The nonparametric test can actually be more powerful in detecting differences among treatment levels, depending on the underlying parametric probability distribution

model.

The output from the program is given in Figures 13-5 through 13-9. Figure 13-5 presents the data on the number of survivors for each treatment, the proportion of survivors, and the

arcsine/square-root transformed proportions. This output was produced by the PROC PRINT; statement in the program in Figure 13-4.

Figure 13-6 presents the arithmetic means and standard deviations of these variables. Note that the number of survivors is more variable (i.e., standard deviations are larger) in the Station treatment groups than in the reference-sediment treatment groups. Note also that the variability among treatment groups is more stable for the transformed survival proportions variable than among the proportions themselves. Output in Figure 13-6 is produced by the PROC MEANS; statement.

Figure 13-7 contains the ANOVA results. These results were produced by the PROC GLM; statement. The *F* value is the statistic of interest in these tables:

$$F = MST/MSE$$

where *MST* is the mean square (variance) for differences among treatment level means (41.1 in this example with NUM_SIV as the dependent variable) and *MSE* is the mean square for differences among replicates (3.18 in this same example). If survival is unaffected by the treatment levels, *F* is approximately equal to 1.0. If survival is less among treatments levels, $F > 1.0$. The probability of obtaining an *F* statistic as large as or larger than the one calculated for the transformed data (i.e., $F = 22.06$) is 0.0001, as given by *Pr* > *F* in the output. That is, if there is no difference in survival among the stations and controls, we would expect to observe survival data like those given in

Table 13-3, only 1 in 10,000. Thus, we reject the hypothesis of equal survival rates at the 0.0001 level of significance.

In this example, there is strong evidence for concluding that there are significant differences in survival among the reference-sediment and dredged-material treatment groups. This conclusion would have been reached whether or not the data are were transformed (Figure 13-7). It is also important to know which sampling stations differed significantly from the reference. The results of an appropriate multiple-comparison analysis known as Dunnett's test (Dunnett, 1964) are given in Figure 13-8. This test was requested in the SAS statements specifying the ANOVA, and the results show that there is no difference in survival between the control group and the reference sediment group either for trans- formed or untransformed data. The negative differences between means and the significance denoted by the asterisks indicate that survival in each dredged-materialtreatment group is signifi- cantly lower than in the reference group. If all the treatment groups (including the reference) actually had the same mean survival, then the probability of concluding that any dredged-material treatment group has a lower mean survival than the reference is 0.05.

The Dunnett's test in the SAS program in Figure 13-4 compares all subsequent treatment groups to the first group in the dataset, that in this case is the reference sediment. *If other software is used, care has to be taken to see that comparisons are made to reference, not control, data.*

Finally, because the number of survivors in each treatment group is not always normally distributed, we have also performed a nonparametric test that does not require the assumption of normality. Figure 13-9 shows the results from a nonparametric Kruskal-Wallis test which was generated by the PROC NPAR1WAY WILCOXON; statement. This test is a counterpart to the parametric ANOVA procedure. It is based on the sum of the ranks for all observations in each treatment group. If survival is consistently lower in the station treatment groups, the sum of the ranks will be smaller. The Kruskal-Wallis statistic is approximately distributed as a chi-square random variable hence, the probability of obtaining as much or more evidence (CHISQ = 19.286) in favor of a difference in survival among the reference and station treatment groups when, in fact, there is no difference is 0.0007, or about 7 times in 10,000. This very small probability is strong evidence that sediments from the proposed dredging site in our hypothetical example truly are more toxic than the reference sediment.

Compliance with the regulations is determined according to the Tier III guidance in Section 6.2.

13.3 BIOACCUMULATION

Bioaccumulation tests described in Section 11 are applied to determine whether an organism's exposure to the dredged material is likely to cause an elevation of contaminants in its body, i.e., is bioaccumulation likely to occur in organisms exposed to the dredged material. Bioaccumulation tests conducted in the laboratory or in the field require statistical analysis as described in Sections 13.3.1 through 13.3.3.

13.3.1 Tier III 10- or 28-Day Single-Time Point Laboratory Study

The Tier III single-time point laboratory bioaccumulation test produces tissue concentration measurements for each contaminant of concern. Table 13-4 presents the results from a hypothetical laboratory test. Chemical analysis of the tissue samples from each replicate shows varying concentrations of the example contaminant.

13.3.1.1 Comparisons with a Reference Sediment

The objective of this type of analysis is to determine whether organisms exposed to the dredged material have a greater bioaccumulation of contaminants than organisms exposed to the reference sediment. One-sided tests are appropriate because there is little concern if the effect of the dredged material is less than the reference sediment.

The ANOVA procedure in Section 13.2.3 is appropriate to use on these data to compare

differences among treatment groups, followed by Dunnett's test to compare individual treatments with the reference sediment. The same type of SAS program as in Figure 13-4 can be used to perform the ANOVA, except that the statement in PROC GLM performing Dunnett's test should be replaced by

means/dunnettu;

This replacement is necessary because we are testing whether any treatment (dredged material at any sampling station) has a larger effect than the reference.

13.3.1.2 Comparisons with an Action Level

In this comparison, the objective is to determine whether the mean bioaccumulation of contaminants in animals exposed to a dredged material is greater than a prespecified action level.

If the dredged material to be used for testing is taken from several dredging stations (e.g., three points within a harbor), then a confidence-interval approach is appropriate.

If the confidence interval for the concentration from a dredged-material exposure contains the FDA level (i.e., the lower confidence interval is less than the FDA level), there is no statistically significant difference between the concentration from the dredged material and the FDA action level (Table 6-1). Conversely, if the FDA level falls below the lower-level confidence interval, the concentration from the dredged material is statistically significantly higher than the FDA action level. One-sided confidence levels are appropriate since there is concern only if the effect in the dredged material is greater than the action level.

The statistics needed for the calculation of confidence levels include the mean and the standard error. These calculations are simple, especially with a small sample size, and can be calculated with paper and pencil. Many calculators include programmed mean and standard-deviation calculations. The sequence of calculations necessary for the statistical analysis is given in the following.

p =Number of stations from which dredged material is taken

n =Number of observations at a particular station

xnj =*nj*th observation, e.g., *x*2 is the second observation

ä*x* =Every *x* summed = *x*1 + *x*2 + *x*3 + . . . + *xn*

ä*x*2 =Every *x* squared = (*x*1)(*x*1) + (*x*2)(*x*2) + . . . + (*xn*)(*xn*)

Mean =ä*x* / *n*

Variance =[ä*x*2 (ä*x*)2/n] / [*n* 1]

Standard deviation = (variance)«

Standard error = standard deviation / standard deviation/(*n*)«

t ,n 1 = 1) quantile of the Student's-*t* distribution with *m* 1 degrees of freedom.

Lower 95%, one-sided confidence level = mean (*t*0.10/*p*,*n* 1)(std. error)

The *t*-distribution resembles the normal distribution in that it is bell-shaped. This distribution, rather than the normal distribution, is used in situations when the population variance of the distribution is not known and is estimated from the sample values. The *t* value to use depends on two parameters: (the probability of a Type I error for a single *t*-test) and the number of degrees of freedom. In the application presented here, the number of degrees of freedom is always one less than the number of observations, i.e., *n* 1. The value of à depends on the probability desired in the tails of the distribution. Here, we are interested in simutaneous 95% one-sided confidence levels; i.e., we want an overall probability of 0.05 of concluding that the mean of at least one of the stations is higher than the action level if, in fact, all of the treatment means are less than the action level. The *t* ,*f* quantiles for various and degrees of freedom *f* are available in most elementary texts on statistics or can be calculated directly by using one of many statistical software packages [e.g., tinv( ) in PC SAS]. Table 13-5 gives an abbreviated *t* distribution table. The *t* value that will give simultaneous 95%, one-sided confidence levels (calculated on for five observations) for the concentrations on each of 3 on each of 5 dredging stations is 3.186 (à = 0.05/3 with *n* 1 = 4 degrees of freedom). Figure 10 shows the relationship of bioaccumulation in the various dredged-material samples to the FDA action level. Average tissue concentration in dredged-material sample number 1 is numerically higher than the FDA action level, whereas the average tissue concentration in dredged-material samples 2 and 3 is below the FDA action level. Bioaccumulation from the dredged material does not statistically exceed bioaccumulation from the reference sediment; i.e., the confidence levels of sample 3 and the reference sediment overlap.

We use simultaneous confidence intervals to control the overall confidence level. If we have *p* dredging stations and place a (1 0.05/*p*) x 100% confidence interval on the average

concentration of each station, then the overall confidence level that all *p* intervals contain the true concentration for their respective stations is at least 95%. Thus, we can draw conclusions on whether each station's true concentration is significantly different from the FDA action level by noting whether the confidence interval contains the FDA level, and our overall conclusion will have an overall Type I error probability of no more than 0.05. If we simply calculated 95% confidence intervals for each station, then the probability of making a Type I error of incorrectly noting a significance between the FDA level and the mean for a station will be higher than 0.05. The simultaneous confidence intervals in Figure 13-10 reflect three stations; thus, each individual

confidence interval is done at the 0.05/3 = 0.017 confidence level. This method of determining simultaneous confidence intervals is known as the Bonferroni method and is discussed by Snedecor and Cochran (1980).

Compliance with the regulations is determined according to the Tier III bioaccumulation guidance in Section 6.3.

13.3.2 Tier IV Time-Series Laboratory Bioaccumulation Study

The 28-day time-series laboratory bioaccumulation test in Tier IV is designed to detect differences, if any, between steady-state bioaccumulation in organisms exposed to the dredged material and steady-state bioaccumulation in organisms exposed to the reference sediment. If organisms are exposed to biologically available contaminants under constant conditions for a sufficient period of time, bioaccumulation will eventually reach a steady state in which maximum bioaccumulation has occurred, and the net exchange of the contaminant between sediment or dredged material and the organism is zero.

A simple kinetic model (McFarland *et al.*, 1986; McFarland and Clarke, 1987) can be used with data collected over a relatively short period of constant exposure to project tissue concentrations at steady state. This model integrated for constant exposure is

$$C_t = \frac{k_1 C_w}{k_2}(1 - e^{-k_2 t})$$

where *Ct* is the concentration of a compound in tissues of an organism at time *t*, *k*1 is the uptake rate constant, *Cw* is the exposure concentration of the compound, *k*2 is the elimination rate constant, and *t* is the time.

As duration of exposure increases, the exponential term in the model approaches zero, and the tissue concentration at steady state (i.e., infinite exposure) is calculated as

$$C_t = \frac{k_1 C_w}{k_2} = C_{ss}$$

where *Css* is an estimate of the whole-body concentration of the compound at steady state (i.e., after infinitely long constant exposure).

Table 13-6 *(11k)* presents tissue concentrations resulting from a hypothetical 28-day time-series laboratory bioaccumulation test on three dredged-material samples. There are five replicates of each treatment, and tissue samples were analyzed on Days 2, 4, 7, 10, 18, and 28 of the test. Mortality in all replicates did not exceed 25%, and therefore the test is acceptable.

These data can be used with iterative nonlinear regression methods such as those in the SAS NLIN procedure to solve for the parameters in the model above. Then *Css*, the steady-state concentration, is simply the ratio of the estimated nonlinear regression parameters *k*1 and *k*2 together with *Cw*. In this iterative calculation method, the contaminant concentration in the

sediment is used as *Cw*. Figure 13-11 provides an SAS/PC program to carry out these calculations. Iterative curve-fitting techniques will provide better fits to some data than to

others. If difficulties are encountered, approaches such as those discussed by SCI (1989) and Draper and Smith (1981) should be considered. The advice of an applied statistician might be appropriate.

Figures 13-12 through 13-17 present the results of the SAS program shown in Figure 13-11. Figure 13-12 is a list of the data used in the program. Figures 13-13 through 13-16 give the nonlinear regression analyses for the reference and dredged materials A, B, and C, respectively. Results of the regression analyses are listed in Figure 13-17.

[Figure 13-12] [Figure 13-13] [Figure 13-14] [Figure 13-15] [Figure 13-16] [Figure 13-17]

In the data listing in Figure 13-12, a value of 999 days is used to represent time infinity at which steady-state concentrations would have occurred.

The confidence levels calculated by the SAS nonlinear regression procedure are 95%, two-sided confidence levels. A one-sided confidence level is calculated from the two-sided levels in the SAS statements in the last data step of the program. The SAS statement incorporate *t* values for

two-sided levels (*t* value: 2.048; *p* level: 0.05 with 28 degrees of freedom) and for one-sided levels [*t* value: 1.701 (Figure 13-12); *p* level: 0.10 with 28 degrees of freedom]. If other than five replicates on each of 6 days (resulting in 30 observations included in the nonlinear regression analysis) are used, these *t* values have to be altered to reflect the correct number of degrees of freedom, which is two less than the total number of observations.

The summary in Figure 13-17 gives the value of the tissue concentration (pre_ct) predicted by nonlinear regression for each day of the test and for steady-state (estimated at 999 days). The summary also includes the corresponding upper and lower 95%, one-sided confidence levels (up_95_1s and lo_95_1s). The predicted steady-state concentrations and their lower confidence levels are compared to FDA action levels and to the upper confidence level calculated on steady- state reference-sediment bioaccumulation.

Figure 13-18 graphically displays the results of the nonlinear regressions of tissue concentration over time for the four treatments. The nonlinear regression line for each treatment is shown with the lower 95% one-sided confidence bounds on the sample means. The regression line and confidence bounds for the reference treatment are solid lines. The lines for treatment A are dotted, for treatment B are dashed, and for treatment C are long and short dashes. Because

bounds have been drawn beyond the time frame of the laboratory test (28 days) to illustrate the steady-state tissue concentration. The hypothetical FDA action level is shown on Figure 13-18 for comparison.

From Figure 13-18, it can be seen that at steady-state bioaccumulation from

dredged-material sample A does not differ from the reference sediment; i.e., the 95% one-sided confidence interval of treatment A overlaps the confidence interval of the reference sediment. At steady-state, the lower bound of sample A is less than the upper bound of the reference sediment. Figure 13-18 also illustrates that the steady state tissue concentration of sample A is less than the FDA action level. For samples B and C, the lower 95% one-sided confidence bounds on concentration at steady state are completely above the confidence bounds of the reference sediment. Since there is no overlap of confidence bounds at steady state, samples B and C differ from the reference sediment at the statistical significance level of 0.05. The mean tissue concentration at steady state for dredged-material sample B is less than the FDA action levels. Steady-state bioaccumulation in sample B is statistically greater than steady-state bioaccumulation in the reference sediment because there is no overlap of confidence levels. The predicted steady-state tissue concentration in dredged-material sample C is not statistically different from the FDA action level, as demonstrated by the lower 95% one-sided confidence bound being lower than the action level.

Compliance with the regulations is determined in accordance with the Tier IV bioaccumulation guidance in Section 7.2.

13.3.3 Steady-State Bioaccumulation from Field Data

The field bioaccumulation test is designed to show differences, if any, between organisms living at the proposed disposal site and organisms living in the sediments in the reference area. This approach is valid only under the conditions described in Section 12.2.2.

The mean tissue concentration in field organisms collected at the disposal site is calculated along with lower 95% one-sided confidence levels using the formulas given in Section 13.3.1. This mean and confidence level are compared to the mean and upper 95% one-sided confidence level calculated at steady state for organisms collected from the reference area. Bioaccumulation in two groups of organisms is considered to be statistically different if the 95%, one-sided confidence intervals do not overlap.

Compliance with the regulations is determined in accordance with Tier IV bioaccumulation guidance in Section 7.2.

13.4 REFERENCES

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14.0 QUALITY-ASSURANCE CONSIDERATIONS

The purpose of a quality-assurance (QA) program in a dredging study is to ensure that the data produced by the study are of known and documented quality. This is accomplished by ensuring that proper quality-control (QC) procedures are built into the study at the beginning and by verifying that the procedures are followed during the study.

The distinction between QA and QC is that the former is a management tool and the latter is a series of procedures designed to implement that tool by measuring precision, accuracy, comparability, completeness, and representativeness. QA activities ensure that QC procedures have been implemented and documented. QA reports to upper management and operates independently of activities involved with conduct of the tests. QC operates as an integral part of the study and includes measurements of data quality, using blanks, spikes, and control test groups to which test results can be compared.

A complete QA effort in a dredging study has two components: a QA program implemented by the responsible governmental agency (the data user) and QA programs implemented by the laboratories performing the tests (the data generators).

14.1 STRUCTURE OF QA PROGRAMS

The organization of the QA effort for a dredging study and the responsibilities of each component are discussed in this section.

14.1.1 Government (Data User) QA Program

The function of the government QA program is to ensure that laboratories contracted for the dredging studies comply with the procedures in this manual or with other specified guidelines. Oversight of the QA effort for a dredging study should be the responsibility of a QA Coordinator to be established in the USACE District Office, working in conjunction with the EPA Regional QA Officer. District QA Coordinators should be responsible for ensuring that data submitted with permit applications and laboratories under contract to their Districts comply with the QA needs of the regulations and guidelines governing dredged-material studies. This responsibility should be carried out in three ways: preaward inspections, interlaboratory comparisons, and routine inspections during conduct of the studies. Data-quality objectives should be established for testing. The QA program should be designed with the assistance of administrative and scientific expertise from Headquarters of EPA and the USACE, and other qualified sources as appropriate. Some QA considerations in contractor selection are discussed by Sturgis (1990).

14.1.1.1 Preaward Inspections

Before a government contract is awarded, it is strongly recommended that the District QA Coordinator inspect the laboratories seeking to work on the study. This preaward inspection assesses the laboratory's capabilities, personnel, and equipment. It establishes the groundwork necessary to ensure that tests will be conducted properly, provides the initial contact between government and laboratory staff, and emphasizes the importance that the government places on quality assurance.

This inspection is designed to establish that the laboratory has implemented the following measures

- An independent QA program
- Written work plans for each test
- Technically sound written standard operating procedures (SOP) for all study activities.

14.1.1.2 Interlaboratory Comparison

In dredging studies it is important for data collected and processed at various laboratories to be comparable. To ensure this comparability, proficiency testing of a laboratory is recommended before a contract is signed and yearly thereafter. Each laboratory taking part in a proficiency test analyzes samples, prepared to a known concentration, of a standard from the National Institute for Standards and Technology (NIST) or other recognized source of standard reference material (SRM) (refer to Table 9-4 for sources of SRMs). Results are compared with predetermined criteria of acceptability. Proficiency testing programs already established by either EPA or the USACE may be used, or a program may be designed specifically for dredging evaluations.

14.1.1.3 Routine Inspections

The purpose of routine surveillance inspections during conduct of contract work is to ensure that laboratories are complying with the QA Plan. It is suggested that the District QA Coordinator develop checklists for review of training records, equipment specifications, QC procedures for analytical tasks, management organization, etc. The QA Coordinator should also establish laboratory review files for quick assessment of the laboratory's activity on a study, and to aid in monitoring the overall quality of the laboratory. Procedures for inspections by the District QA Coordinator are similar to systems audits (Section 14.3.4) conducted by the laboratories themselves.

14.1.2 Data Generator QA Program

Ideally, each laboratory participating in a dredged-material study should have a written QA Program Plan that describes the organization's QA program, including its policies, areas of application, and authorities. Individuals involved in the QA program should be identified and their responsibilities clearly stated. For any given study, QA personnel

should be entirely independent of the technical personnel engaged in the study to ensure unbiased assessments of the work performed.

Where possible, the laboratory should have a QA Manager or Coordinator who is responsible for the development, implementation, and administration of the QA program. For dredging studies, the QA Manager/Coordinator should ensure that the appropriate QA planning documents exist for each study (Section 14.2.8); routine procedures that impact data quality are described in SOPs; sufficiently detailed audits are conducted at intervals frequent enough to ensure conformance with approved study plans and SOPs and to identify deficiencies; and appropriate corrective actions are implemented in a timely manner.

14.2 GENERAL COMPONENTS OF ALL QA PROGRAMS

A well-structured QA program defines the criteria that the data must meet to be acceptable. The procedures for collecting and analyzing those data should be an integral part of the overall study plan. A good QA program sets standards for personnel qualifications, facilities, equipment, services, data generation, recordkeeping, and data-quality assessments.

14.2.1 Organization

The QA program plan should describe the lines of authority and responsibilities for technical personnel, including those responsible for quality assurance. Procedures should be in place for describing the qualifications, training, job descriptions, etc., for all field and laboratory personnel.

14.2.2 Personnel Qualifications

All personnel performing tasks and functions related to data quality have to be appropriately qualified and adequately trained. It is generally the responsibility of the contractor's QA staff to ensure that personnel are qualified and trained. Records of qualifications and training of personnel should be kept current so that training can be verified by internal QA personnel or by EPA and the USACE.

14.2.3 Facilities

The QA program plan should provide a description of the physical layout of the laboratory, define space for each area of testing, describe traffic-flow patterns, and document special laboratory needs.

14.2.4 Equipment and Supplies

The QA program plan should describe how field and laboratory equipment essential to the performance of environmental measurements will be maintained in proper working

order. This is demonstrated through records that document the reliability and performance characteristics of the equipment. Such equipment should be subject to regular inspection and preventive-maintenance procedures to ensure proper working order. Instruments should have periodic calibration and preventive maintenance performed by qualified technical personnel, and a permanent record kept of calibrations, problems diagnosed, and corrective actions applied. An acceptance testing program for key materials used in the performance of environmental measurements (chemical and biological materials) should be applied prior to their use.

14.2.5 Test Methods and Procedures

All methods and procedures used in the field and laboratory should be in written form, authorized, and readily available to all personnel. There should be a mechanism to describe the circumstances under which nonstandard methods or procedures may be used, and the appropriate approval and documentation should be described.

14.2.6 Sample Handling and Tracking

Sample custody is a part of any good field or laboratory operation. Where samples may be needed for potential litigation, chain-of-custody procedures should be used. Sample custody is important for both parts of the dredged-material evaluation process the field (sample collection) and the laboratory (receipt, analysis and reporting). More detailed sample-handling guidance is provided in Sections 8.2.6 through 8.2.8.

14.2.7 Documentation and Recordkeeping

Records should be maintained to ensure that all aspects of the field and laboratory work are documented. It is important to record all the events that are associated with a sample so that the scope and validity of the resulting data may be properly interpreted. A document trail is generated to show the course of the sample from the field through the laboratory.

All data should be recorded directly, promptly, legibly, and indelibly, so that data are easily traceable. Data entries should be dated on the date of entry and signed or initialed by the person making the measurement and the person entering the data. Changes on entries should be made so as not to obscure the original entry, and should indicate the reason for the change, the person making the change, and the date of change. In computer-driven data-collection systems, the person responsible for direct data input should be identified at the time of input.

14.2.8 Quality-Assurance Plan

It is good practice for the government to require that QA study plans be developed by the contractor for all dredged-material evaluations. These study plans may be developed in accordance with either EPA (1984) or the USACE (1985). EPA (1987) contains QA guidance that is generally applicable to sample collection and laboratory aspects of

dredged-material evaluations and should be considered in QA study-plan development. Topics covered in these documents include provisions for (1) name of the study, (2) name of requesting agency, (3) date of the request, (4) date of initiation, (5) program officer, (6) QA officer, (7) study description, (8) fiscal information, (9) schedule of tasks and products, (10) organization and responsibilities, (11) data- quality requirements and assessments, (12) sampling and analytical procedures, (13) sample- custody procedures, (14) equipment calibration and maintenance procedures, (15) documentation, data reduction, and reporting, (16) data validation, (17) performance and systems audits, (18) corrective action, and (19) reports. QA study plans are valuable documents because they provide in one place an overall plan for conducting work, including standards of data quality that have to be maintained. QA study plans are particularly useful for work that involves many people or that lasts over a long period. When many people are involved, the plan ensures that everyone has a thorough understanding of the goals and procedures of the program. When work is conducted over a long period, the plan provides a basis of continuity, ensuring that procedures do not slowly change over time without the persons involved in the program evaluating the nature of the changes and their possible impact on data quality.

14.2.9 Standard Operating Procedures (SOP)

Standard operating procedures (SOP) are documents describing routine study methods and procedures that affect data quality and integrity. Like QA study plans, SOPs ensure that all persons conducting work are following the same procedures and that the procedures do not change over time. SOPs should be prepared for use of equipment and facilities, measurements, and other aspects of work that impact data quality.

14.3 DATA-QUALITY ASSESSMENT

14.3.1 Data Validation

Data validation involves all procedures used to accept or reject data after collection and prior to use, including editing, screening, checking, auditing, verifying, and reviewing. Data- validation procedures ensure that the standards for data accuracy and precision were met, that data were generated in accordance with the QA study plan and SOPs, and that data are traceable and defensible. It is important for all reported data to be properly validated following standardized procedures to ensure that data are of consistent and documented quality.

14.3.2 Chemical Quality Control

Chemical QC specifications are the ranges considered acceptable for instrument calibration, analyte recovery, data accuracy, and data precision. Instrument calibration involves determining a linear response over the range of data to be collected. Recovery is determined by analyzing a sample spiked with a known amount of chemical. Procedural

accuracy is established by including a series of spiked and blank samples in each analysis. Precision is established by analyzing replicate samples. QC procedures are discussed in more detail for sediment, water, and tissue analyses in Sections 9.3.3, 9.4.3, and 9.5.3, respectively.

The USACE District QA Coordinator or management authority for the program may require that certain samples be submitted on a routine basis to government laboratories for analysis, and EPA or the USACE may participate in some studies. These activities provide an independent quality assurance check on activities being performed and on data being generated.

14.3.3 Biological Quality Control (Reference-Toxicant Testing)

Biological QC involves periodic reference-toxicant tests conducted with all stocks of organisms to be used in the dredged-material tests to determine the relative health of the test organisms. The application and benefits of reference-toxicant tests are discussed by Lee (1980). Detailed assistance in establishing a biological QC program can be provided by scientists from Headquarters of EPA and the USACE. When sufficient reference-toxicant data have been generated for a particular species, it may be possible to stipulate an acceptable LC50 range for that species with the reference toxicant.

14.3.4 Performance and System Audits

Performance and system audits are an essential part of the field and laboratory QA program. A performance audit independently collects measurement data using performance evaluation (PE) samples, field blanks, trip blanks, duplicate samples, and spiked samples. A systems audit consists of a review of the total data production process that includes on-site reviews of field and laboratory operational systems. The purpose of these inspections is to verify that (1) appropriate SOPs are in place, (2) training of the staff is appropriate and documented, (3) all equipment is properly calibrated and maintained, (4) approved analytical procedures are being followed, and (5) all aspects of the study are on schedule.

14.3.5 Management of Nonconformance Events

One purpose of any QA program is to identify a nonconformance event as quickly as possible. A nonconformance event is defined as any event that does not follow defined methods, procedures, protocols, or any occurrence that may affect the quality of the data or study. A QA program should have a corrective action plan to provide feedback channels to the appropriate management authority defining how all nonconformance events were corrected.

14.3.6 Archiving of Data and Samples

A procedure should be established for the retention of all appropriate field and laboratory records, specimens, and samples as various tasks or phases are completed. The archiving

procedure should indicate the storage requirements, location, indexing codes, retention time, security, and environmental measures needed to preserve the data and samples.

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B1.0 INTRODUCTION

This appendix presents guidance for the use of numerical models for evaluation of mixing as part of the Tier II and Tier III water-column evaluations. The versions of the models in this appendix are a part of the Automated Dredging and Disposal Alternatives Management System (ADDAMS) (Schroeder and Palermo, 1990) and can be run on a personal computer (PC). ADDAMS is an interactive computer-based design and analysis system in the field of dredged-material management. The general goal of the ADDAMS is to provide state-of-the-art computer-based tools that will increase the accuracy, reliability, and cost-effectiveness of dredged-material management activities in a timely manner.

B1.1 MODEL APPLICATIONS

Any evaluation of potential water-column effects has to take into consideration the effects of initial mixing. Section 227.29 defines initial mixing as follows.

> *Initial mixing is defined to be that dispersion or diffusion of liquid, suspended particulate, and solid phases of a waste which occurs within four hours after dumping. The limiting permissible concentration* [LPC] *shall not be exceeded beyond the boundaries of the disposal site during initial mixing, and shall not be exceeded at any point in the marine environment after initial mixing.*

Versions of the models described in this appendix, for use on IBM-compatible microcomputers, are provided on the diskettes in the pocket inside the back cover of this manual. The diskettes contain models appropriate for three types of discharges that may be used for ocean dumping instantaneous discharges, continuous discharges, and hopper-dredge discharges. The user must select the appropriate model for the particular disposal operation proposed. Each of these three types of discharge model described in this appendix has been designed to evaluate initial mixing for each of the three specific applications described in this manual. As discussed in the remainder of Section B1.1, these applications, which are progressively more precise and should be used sequentially, are

- **Model application for screen to determine WQC compliance in Tier II**

  In this application of the model, the dredged material is screened for potential impact by conservatively assuming that all contaminants in the dredged material are available to water-column organisms. This application is based on whole-sediment contaminant concentrations.

- **Model Application for Elutriate Analysis To Determine WQC Compliance in**

Tier II

In this application of the model, measured concentrations of contaminants in an elutriate of the dredged material are used to evaluate the potential for water-column impact at the disposal site. The elutriate data provide a more accurate determination of impact than those which can be obtained by using the whole-sediment data that are used in the screen.

- **Model Application for Water-Column Bioassays in Tier III**

  In this application of the model, the potential for water-column impact is further described by using the model to relate biological test results to contaminant concentrations that could occur at the disposal site.

B1.1.1 Model Application for Screen to Determine WQC Compliance in Tier II

The evaluation of the potential for water-column impact in Tier II begins with a determination of the necessity of additional water-column testing. This determination is based on a standardized calculation comparing contamination of the dredged material with WQC, considering the effects of initial mixing. The models need be run only for the contaminant requiring the greatest dilution to meet its WQC. It should be noted that contaminant concentration in dredged material usually is expressed in micrograms per kilogram (æg/kg) dry weight. The model uses contaminant concentration in micrograms per liter (æg/L) when calculating the necessary dilution factor for the dredged material (Section 10.1.1). To convert the contaminant concentration reported on a dry-weight basis to the contaminant concentration in the dredged material, the dry-weight concentration must be multiplied by the mass of dredged-material solids per liter of dredged material.

The key parameters derived from the dispersion models are the maximum concentration of the contaminant in the water column outside the boundary of the disposal site during the 4-h initial-mixing period, and the maximum concentration anywhere in the marine environment after the 4-h initial-mixing period. These concentrations are compared with the applicable marine WQC according to the guidance in Section 10.1.1 to determine if additional water-column testing is necessary.

B1.1.2 Model Application for Elutriate Analysis To Determine WQC Compliance in Tier II

If additional water-column testing is necessary, the potential for water-column impact should be evaluated under Tier II by comparing predicted dissolved contaminant concentrations in the standard elutriate (in micrograms per liter) (Section 10.1.2) with the WQC, considering the effects of initial mixing. The models need be run only for the contaminant requiring the greatest dilution to meet its WQC. The key parameters derived from the models are the maximum dissolved concentration of the contaminant outside the boundary of the disposal site during the 4-h initial-mixing period, and the maximum

concentration anywhere in the marine environment after the 4-h initial-mixing period. This concentration is compared to the applicable marine WQC according to the guidance in Section 10.1.2.3 to determine if the discharge is acceptable.

B1.1.3 Model Application for Water-Column Bioassays in Tier III

If there are no WQC for all contaminants of concern or if synergistic effects are suspected, the potential for water-column impact should be evaluated under Tier III by comparison of predicted concentrations of the suspended plus dissolved constituents of the dredged material (in percent) with bioassay results, considering the effects of initial mixing (Section 11.1). For this case, the models calculate the dilution of the dredged material expressed as a percent of the initial concentration. The key parameters derived from the model are the maximum concentration of dredged material in the water column outside the boundary of the disposal site during the 4-h initial-mixing period, and the maximum concentration anywhere in the marine environment after the 4-h initial-mixing period. These concentrations are compared to 0.01 of the LC50 as determined by the bioassay tests according to the guidance in Section 11.1.7 to determine if the discharge is acceptable.

B1.2 MODEL DESCRIPTIONS AND LIMITATIONS

The models account for the physical processes determining the short-term fate of dredged material disposed at open-water sites. The models provide estimates of water-column concentrations of dissolved contaminants and suspended sediment and the initial deposition of material on the bottom.

Two of the models were developed by Brandsma and Divoky (1976) under the United States Army Corps of Engineers (USACE) Dredged Material Research Program to handle both instantaneous dumps and continuous discharges. The models were based on work by Koh and Chang (1973). A third model that utilized features of the two earlier models was constructed later to handle a semicontinuous disposal operation from a hopper dredge. These models are known as DIFID (Disposal from an Instantaneous Dump), DIFCD (Disposal from a Continuous Discharge), and DIFHD (Disposal from a Hopper Dredge). Collectively, the models are known within ADDAMS as the Open Water Disposal (DUMP) Models.

For evaluation of initial mixing for ocean disposal, the models need be run only for the contaminant requiring the greatest dilution to meet its WQC. A data-analysis routine is contained in the models for calculating the required dilutions and determining which contaminant should be modeled.

In all three models, the behavior of the material is assumed to be separated into three phases: (1) convective descent, during which the dump

cloud or discharge jet falls under the influence of gravity and the initial momentum of the

discharge; (2) dynamic collapse, occurring when the descending cloud or jet either impacts the bottom or arrives at a level of neutral buoyancy where descent is retarded and horizontal spreading dominates; and (3) passive transport and dispersion, commencing when the material transport and spreading are determined more by ambient currents and turbulence than by the dynamics of the disposal operation.

These models simulate movement of the disposed material as it falls through the water column, spreads over the bottom, and finally is transported and diffused by the ambient current. DIFID is designed to simulate the movement of material from an instantaneous dump that falls as a hemispherical cloud. Thus, the total time required for the material to leave the disposal vessel should not be greater than the time required for the material to reach the bottom. DIFCD is designed to compute the movement of material disposed in a continuous fashion at a constant discharge rate. Thus, it can be applied to pipeline disposal operations in which the discharge jet is below the water surface or perhaps to the discharge of material from a single bin of a hopper dredge. If the initial direction of disposal is vertical, either the disposal source has to be moving or the ambient current has to be strong enough to result in a bending of the jet before the bottom is encountered. DIFHD has been constructed to simulate the fate of materials disposed from stationary hopper dredges. Here, the normal mode of disposal is to open first one pair of doors, then another, etc., until the complete dump is made, which normally takes on the order of a few minutes to complete. DIFHD should not be applied to disposal operations that differ significantly from that described above.

In addition, it should be noted that the disposed material is expected to behave as a dense liquid. This will be true only if the material is composed of primarily fine-grained solids. Thus, the models should not be applied to the disposal of purely sandy material. A major limitation of these models is the basic assumption that once solid particles are deposited on the bottom, they remain there. Therefore, the models should be applied only over time frames in which erosion of the newly deposited material is unimportant.

The passive transport and diffusion phase in all three models is handled by allowing material settling from the descent and collapse phases to be stored in small Gaussian clouds. These clouds are then diffused and transported at the end of each time step. Computations on the long-term grid are made only at those times when output is desired.

The use and limitations of the models along with theoretical discussions are presented in detail by Johnson (1990). Additional technical references for the models are provided in the bibliography of this appendix and online in the system. Their review is strongly recommended.

B1.4 MODEL INPUT

Input data for the models are grouped into the following general areas: (1) description of the disposal operation, (2) description of the disposal site, (3) description of the dredged materials, (4) model coefficients, and (5) controls for input, execution, and output.

Ambient conditions include current velocity, density stratification, and water depths over a computational grid. The dredged material is assumed to consist of a number of solid fractions, a fluid component, and conservative contaminants. Each solid fraction has to have a volumetric concentration, a specific gravity, a settling velocity, a void ratio for bottom deposition, and information on whether or not the fraction is cohesive. For initial-mixing calculations, information on initial concentration, background concentration, and WQC for the constituent to be modeled has to be specified. The description of the disposal operations for the DIFID model includes the position of the disposal barge on the grid, the barge velocity, and draft, and volume of dredged material to be dumped. Similar descriptions for hopper dredge and pipeline operations are required for the DIFCD and DIFHD models. Coefficients are required for the models to accurately specify entrainment, settling, drag, dissipation, apparent mass, and density-gradient differences. These coefficients have default values that should be used unless other site-specific information is available. Table B-1 *(27k)* lists the necessary input parameters with their corresponding units. More detailed descriptions and guidance for selection of values for many of the parameters is provided directly online in the system.

B1.5 MODEL OUTPUT

The output starts by echoing the input data and then optionally presenting the time history of the descent and collapse phases. In descent history for the DIFID model, the location of the cloud centroid, the velocity of the cloud centroid, the radius of the hemispherical cloud, the density difference between the cloud and the ambient water, the conservative constituent concentration and the total volume and concentration of each solid fraction are provided as functions of time since release of the material. Likewise, the location of the leading edge of the momentum jet, the centerline velocity of the jet, the radius of the jet, the density difference between material in the jet and the ambient water, the contaminant concentration, and the flux and concentration of each solid fraction are provided as functions of time at the end of the jetconvection phase in DIFCD and DIFHD.

At the conclusion of the collapse phase in DIFID and DIFHD, time-dependent information concerning the size of the collapsing cloud, its density, and its centroid location and velocity as well as contaminant and solids concentrations can be requested. Similar information is provided by DIFCD at the conclusion of the jet-collapse phase. These models perform the numerical integrations of the governing conservation equations in the descent and collapse phases with a minimum of user input. Various control parameters that give the user insight into the behavior of these computations are printed before the output discussed above is provided.

At various times, as requested through input data, output concerning suspended sediment concentrations can be obtained from the transport-diffusion computations. With Gaussian cloud transport and diffusion, only concentrations at the water depths requested are

provided at each grid point.

For evaluations of initial mixing for ocean disposal, results for water-column concentrations can be computed in terms of milligrams per liter of dissolved constituent for Tier II evaluations or in percent of initial concentration of suspended plus dissolved constituents in the dredged material for Tier III evaluations. The maximum concentration within the grid and the maximum concentration at or outside the boundary of the disposal site are tabulated for specified time intervals. Graphics showing the maximum concentrations inside the disposal-site boundary and anywhere on the grid as a function of time can also be generated.

B1.6 GENERAL INSTRUCTIONS FOR RUNNING THE MODELS

B1.6.1 Target Hardware Environment

The system is designed for the IBM PC-AT (including compatibles) class of personal computers. This does not constitute official endorsement or approval of these commercial products. In general, the system requires a mathematics coprocessor, 640 kb of RAM and a hard disk. The models are written primarily in Fortran 77 but some of the higher-level operations and file-management operations are written in BASIC and some of the screen control operations in the Fortran 77 programs are performed using an Assembly language utility program.

B1.6.2 Installation and Starting

All files contained on the diskettes in the folder in the back of this manual should be saved in a directory on the hard disk dedicated for the ADDAMS system, e.g. C:\ADDAMS. The files are archived on the diskettes and have to be dearchived prior to running the models. To dearchive the files, copy the files from each diskette onto the hard drive, call up the README file, and follow the instructions.

B1.6.3 User Interface

The models in the DUMP application of ADDAMS employ a menu-driven environment with a full-screen data-entry method. In general, single keystrokes (usually the F1 through F10 function keys, the number keys, Esc key or the arrow keys and the Enter key) are required to select menu options in the system. Menus are displayed on the screen. Cursor keys are used to select from among highlighted input fields (displayed in reverse video) much like a spreadsheet program. To enter alphanumeric data, the user moves the cursor to the cell of interest, using the up and down arrows to move, respectively, up and down, the Tab and Shift-Tab keys to move, respectively, right and left. The Enter key is also used to move forward through the cells. The left and right arrow keys are used to move the cursor within a selected cell to edit the cell's contents.

The Backspace key is used to delete a single character in a cell. The Delete and Insert keys are used to delete and insert a row of data on a screen of tabular data. Using the PgDn key causes the cursor to move to the next data-entry screen and the PgUp key to move to the previous data-entry screen. The Esc key permits the user to quit data entry on the present operation and to exit to the previous menu. The Home key permits the user to exit from the current data-entry screen to the Main Menu for the application, without loss of data. Results from computations are generally displayed in tabular format on the screen and/or written to print files or devices.

B1.7 STEPS IN USING THE MODEL

The basic steps to follow in applying the models within their menu-driven environment are illustrated in Figure B-1. The general steps and the corresponding menus used in applying the model for a disposal operation are as follows.

a. *Starting*. Change the directory to make the ADDAMS directory the default directory. Start the program by entering ADDAMS at the prompt. The program will display first the ADDAMS logo and then an Application Selection Menu. An application in the ADDAMS software consists of one or more standalone computer programs or numerical models for performing a specific analysis. The only ADDAMS application provided on diskette with this manual is named DUMP. DUMP consists of programs for evaluating open-water disposal of dredged material. Select the DUMP application from the Application Selection Menu. This causes the program to display a File Manager Menu for the DUMP application input data files.

b. *File manager menu*. At this point, an input data file or DOS path for data storage may be selected or named. An existing input data file may be selected by displaying a directory of data files on the specified DOS path. Other file-management operations may also be performed on input data files. Input data file names are given an extension of .DUI by the program. After completing all file-management operations, if any, select the option to continue. The program will display a reference screen with points of contact and then the DUMP Activity Selection Menu.

c. *Activity selection menu*. The activity selection menu may be considered the main menu for the DUMP application. The first option is used to analyze bulk-sediment and elutriate data for determining which specific contaminant should be selected for modeling (see step d). The second option is used to enter data and build, edit, or write input and execution data files (see step e). The third option executes the simulation and graphics (see step k), and the remaining options print or review output files and graphics (see step l).

d. *Dilution Requirements for Initial Mixing Menu*. A data-analysis routine controlled by this menu is used to select a specific contaminant for modeling. Such a selection is necessary under the Tier II analysis both for evaluation of the need for additional testing and for water-quality comparisons with criteria. Execution of the open-water disposal

models for these Tier II analyses allow use of only one contaminant; this option is used to select that contaminant. Bulk sediment contaminant concentrations and WQC are required to compute the required dilutions for the evaluation of the need for additional testing. The contaminant requiring the largest dilution should be subsequently modeled.

Elutriate and background concentrations and WQC are required to compute the required dilutions for the analysis to compare dissolved contaminant concentrations with WQC. The contaminant requiring the largest dilution should be subsequently modeled.

e. *Disposal-Type Selection Menu*. The selection of a disposal type under this menu controls the input data requests, the type of execution data file that will be built, and the open-water disposal model that will be executed. Select the appropriate type of disposal: Disposal from a Hopper Dredge, Continuous Discharge from a Pipeline, or Instantaneous Dump from a Barge or Scow. The input data file last used by the program or selected earlier in step b will be read. If the file is new, the input data will be initialized. A DUMP Input Activity Selection Menu will then be displayed.

f. *Input Activity Selection Menu*. The first option is used to read a different input data file or initialize a new data file. This option will call the DUMP Input File Manager Menu to permit file selection (see step g for description). After selecting or initializing an input data file, if needed, select the second option to enter or edit input data and write input and execution data files. A DUMP Input Selection Menu will be displayed.

g. *Activity File Manger Menu*. A similar file manager is used for input, execution, or output data file selection and saving. The first option is used to specify the name of the file to be used (saved, read, viewed, plotted, or printed). The file specified in this option becomes the active data file. If needed, the second option is used to specify the DOS path to the location where the data file should be read or saved. The third option displays a directory of appropriate DUMP data files for the current path. An existing data file name may be selected from the list to use as the active data file name for overwriting or reading existing data. For example, one option may save the existing data in a file having the active data file name. The other options available are dependent on the routine (menu option) calling the file manager. The input data that are stored in files with an extension of .DUI are displayed in the input data screens displayed under this option. This option is used also to build execution data files. Execution data files are the actual input data files used by the open-water disposal model to perform the analysis and generate output. These files are unique in structure to the input requirements of a particular open-water disposal model, either DIFHD, DIFCD or DIFID. The files are stored with an extension of .DUE. Other call/dependent options include starting the reading, viewing, or graphics.

h. *Input Selection Menu*. Five types of input data have to be entered, plus any desired changes in the default set of model coefficients, before an execution data file can be written. Default values are included for all of the model coefficients requested. An input data file may be written at any point to save all the data that have been entered up that point. Enter data by paging down through the data-entry screens and filling in the cells for each option.

i. *Write input data file*. Write an input data file to save the input data for future editing and use of the appropriate option under the DUMP Input Selection Menu. A DUMP Activity File Saving Menu will be displayed (see step g).

j. *Write execution data file*. Write an execution data file to save the input data in the data structure used by the selected open-water disposal model. The execution data file is the input used during execution of the simulation. This is performed by selecting the appropriate option on the DUMP Input Selection Menu. A DUMP Activity File Saving Menu will be displayed (see step g). All steps required for data entry or editing have been completed and the program is ready to execute the analysis.

k. *Execute*. Return to the DUMP Activity Selection Menu by repeatedly pressing the Esc key. Select the option to execute the open-water disposal model. This option uses an execution data file to generate an output file and graphics file of the same name as the execution data file selected but with an extension of .DUO and .DUP, respectively, instead of .DUE. An Execution Data File Selection Menu will be displayed that is similar to the file-manager menu described in step g. The only difference is that an option is provided to execute the disposal model instead of saving and writing the data file. The program will then execute the analysis using the selected execution data file and generate output and graphics files. Depending on the structure of the execution data file, either the DIFHD, DIFID, or DIFCD model will be executed. The execution may take a few minutes or several hours, depending on the simulation selected and the computer hardware used, but typically 30 min is sufficient. For long-term transport diffusion computations the DIFCD program may require about 5 times as long to run as the other disposal models.

l. *Print, View, or Plot Results*. To display the results, select the appropriate option on the DUMP Activity Selection Menu. A DUMP Output or Graphics Data File Selection Menu will be displayed that is similar to the file-manager menu described in step g. The only difference is that an option may be selected to display the output. The output has 132 characters per line and should be printed using compressed print or wide paper. The program will automatically use compressed print on some printers, mainly Epson and IBM printers. It may be necessary to turn on compressed printing on your printer prior to printing the output, or to print the output outside the ADDAMS program, using the DOS print command or a word processor. In addition, the DUMP Output Data File Selection Menu has an option to view the output using the LIST.COM utility program. Similar options are available to view graphic output. This step completes execution of the DUMP application.

m. *Ending*. To exit the program, press Esc repeatedly until you obtain a DOS prompt. During execution of a particular application's program, the user has to wait until the sometimes lengthy computations are computed. The program can also be terminated by a Control-Break or by turning off the computer, but loss of data may occur. These methods of ending are not recommended. Similar methods are available during printing of output.

B1.8 EXAMPLE APPLICATIONS

Three example applications are presented in this appendix. The examples illustrate the use of DIFID to evaluate for the need for additional water-column testing (Tier II), DIFCD for a comparison of dissolved contaminant concentrations with WQC (Tier II), and DIFHD for comparison of water-column concentrations of dredged material with bioassay results (Tier III). Descriptions of the examples and a discussion of the model results follow. The input and output files for each of the examples are saved on the diskettes in the pocket in the back of this manual.

B1.8.1 Example Application of DIFID

This example demonstrates the application of the instantaneous dump model DIFID and the evaluation of the need for additional water-column testing under Tier II. The input and output files for this example are named DIFID.DUI and DIFID.DUO, respectively.

B1.8.1.1 Operations Information

Disposal from a split hull barge at a disposal site with a constant water depth is modeled. The total volume of the dredged material is 1000 cu yd and is contained in a barge 100 ft long and 50 ft wide. The barge is stationary at the point of release. The unloaded draft of the barge is 5.0 ft, and the time required to empty the barge is 5.0 s.

B1.8.1.2 Disposal-Site Information

The disposal site is 6000 6000 ft. A 30 30 grid with a 1500-ft grid spacing was selected, with the disposal site centered in the grid. The total water depth is 100 ft and there is no bottom slope. The ambient water current is 2.0 ft/s, directed from south to north for the upper 40 ft of the water column. The current then reverses direction over the next 20 ft to become 2.0 ft/s, directed from north to south at a depth of 60 ft below the surface. A linear decrease to a value of zero at the bottom follows. The ambient density profile is a constant 1.018 g/c3 from the surface to depth of 40 ft, increasing to 1.022 g/c3 at a depth of 60 ft, and a constant of 1.022 g/c3 to the bottom.

B1.8.1.3 Dredged-Material Information

The dredged material is composed of a sand and a silty-clay solid fraction. The sand volumetric concentration is 0.14 ft3/ft3 and silty-clay volumetric concentration is 0.17 ft3/ft3. The remaining 0.69 ft3/ft3 is composed of water (both void spaces and entrained water). The settling velocity of the sand is taken to be 0.07 ft/s, whereas the silty-clay fraction is treated as a cohesive fraction with the settling velocity internally computed. Following deposition on the bottom, a void ratio of 4.0 is specified for the silty-clay fraction, whereas a void ratio of 0.8 is specified for the sand. The required dilutions of all contaminants of concern to meet their respective WQC were computed. Cadmium was found to be the contaminant of concern, requiring the highest dilution to meet its WQC, and was selected as the parameter to be modeled for evaluation of the need for additional

water-column testing. The sediment concentration for cadmium is 20 mg/kg and the acute marine WQC for cadmium is 0.043 mg/L .

B1.8.1.4 Coefficients

Default values were used for all coefficients.

B1.8.1.5 Controls for Execution and Output

The total simulation time is specified as 4 h or 14,400 s, with a 600-s computational time step. Output is specified for depths of 10, 50, and 99 ft, which correspond to near surface, mid-depth and near bottom, respectively.

B1.8.1.6 Summary of Output

As can be seen from the output, the disposal cloud strikes the bottom in 7.19 s and grows from an initial radius of 23.44 ft to a final radius at the bottom encounter of 47.58 ft. Collapse on the bottom then occurs, with the collapse phase terminated at 32.62 s after the disposal, with the final cloud having a diameter of 1234.98 ft. During the initial-mixing period of 4 h, the calculated maximum concentration of cadmium outside the disposal-site boundary is 0.000682 mg/L, occurring 40 min after disposal at a depth of 50 ft. This concentration is less than the acute WQC of 0.043 mg/L. Therefore, there is no need for additional water-column testing according to the guidance in Sections 10.1.1 and 5.1.

B1.8.2 Example Application of DIFCD

This example demonstrates the application of the continuous-discharge model DIFCD and the comparison of dissolved contaminant concentrations with WQC under Tier II. The input and output files for this example are named DIFCD.DUI and DIFCD.DUO, respectively.

B1.8.2.1 Operations Information

A pipeline disposal operation from a stationary barge at a disposal site with constant water depth of 50 ft is modeled. The pipeline is 1.0 ft in diameter with a discharge rate of 5 ft3/s for 3600 s. The end of the pipe is located at a water depth 10 ft below the surface at an angle of 90ø with respect to the water surface.

B1.8.2.2 Disposal-Site Information

The disposal site is 3000 3000 ft. A 30 30 grid with a 250-ft grid spacing was selected. The disposal site is located within one corner at a distance of 2250 ft from the northern edge of the grid and 500 ft from the western edge of the grid and with the opposite corner 5250 ft from the northern edge of the grid and 3500 ft from the western edge of the grid. The discharge point is located 4000 ft from the northern edge of the grid and 1500 ft from

the western edge of the grid. The disposal site is a constant-depth site of 50 ft. The ambient-water current is directed from west to east, with a magnitude of 0.5 ft/s over the upper 45 ft of the water column. The velocity then linearly decreases to 0.25 ft/s at 1 ft above the bottom and finally to zero at the bottom. The ambient density is assumed to vary linearly from 1.0 g/c3 at the surface to 1.010 g/c3 at the bottom.

B1.8.2.3 Dredged-Material Information

The dredged material is a slurry with an average bulk density of 1.32 g/c3 and is composed of two solid fractions, sand and silt. The concentration of each is 0.10 ft3/ft3. The settling velocity is 0.07 ft/s for sand and 0.02 ft/s for silt. The void ratio after bottom deposition is 3.0 for silt and 0.8 for sand. A previous evaluation indicated a need to conduct additional water-column testing. Tests were performed to determine initial dissolved contaminant concentrations in the water column under Tier II. The required dilutions of all contaminants of concern to meet their respective WQC were computed. Cadmium was found to require the highest dilution and was selected as the parameter to be modeled and compared with its WQC. The initial water-column concentration of dissolved cadmium was determined to be 0.9 mg/L, the background concentration for cadmium was 0.001 mg/L, and the acute marine WQC for cadmium is 0.043 mg/L.

B1.8.2.4 Coefficients

Default values were used for all coefficients.

B1.8.2.5 Controls for Execution and Output

The total simulation time is specified as 4 h or 14,400 s, with a 900-s computational time step. Output is specified for depths of 30 and 49 ft, which correspond to middepth and near bottom, respectively.

B1.8.2.6 Summary of Output

As indicated in the output, the momentum jet strikes the bottom after 10.29 s, with a radius of 4.496 ft. Collapse on the bottom terminates after 29.66 s. The calculated maximum concentration of cadmium after the 4-h initial-mixing period is 0.000013 mg/L above background, and the maximum concentration of cadmium outside the disposal site boundary during the 4-h initial-mixing period is 0.0002 mg/L above background. Both of these values are less than the WQC of 0.043 mg/L, and are acceptable according to the guidance in Sections 10.1.2.3 and 5.1.2.

B1.8.3 Example Application of DIFHD

This example demonstrates the application of the hopper-dredge model DIFHD and the comparison of water-column concentrations of dredged material with water-column bioassay results under Tier III. The input and output files for this example are named DIFHD.DUI and DIFHD.DUO, respectively.

B1.8.3.1 Operations Information

A disposal operation is modeled from a stationary hopper dredge containing eight bins configured in four pairs of two bins, with pairs of bins opened sequentially. Disposal is assumed to occur from pairs of bins with the disposal from one pair essential complete before the disposal from the next pair begins. The total discharge takes 120 s and occurs through bin doors with a cross-sectional area of 16 ft2, which yields an equivalent circular geometry with a radius of 2.26 ft. The centerline distance between the bins is 14 ft. The loaded draft is 10 ft. The discharge rate from each bin is taken to be 75 ft3/s.

B1.8.3.2 Disposal-Site Information

The disposal site is 5250 5250 ft. A 30 30 grid with a 750-ft grid spacing was selected. The disposal site is located within the grid with one corner at a distance of 8250 ft from the northern edge of the grid and 2250 ft from the western edge of the grid and with the opposite corner 13,500 ft from the northern edge of the grid and 7500 ft from the western edge of the grid. The location of the hopper dredge is 4500 ft from the western edge of the grid and 11,250 ft from the northern edge of the grid. The disposal site is a constant depth site with a water depth of 75 ft and no bottom slope. The ambient current is 0.9 ft/s over the upper 70 ft of the water column and is directed from west to east. The velocity then decreases linearly over the next 4 ft to 0.2 ft/s, then linearly over the next foot to zero. The ambient density is 1.00 g/c3 at the surface and increases linearly to 1.01 g/c3 at the bottom.

B1.8.3.3 Dredged-Material Information

The dredged material is composed of sand and clay solid fractions, each having a concentration of 0.10 ft3/ft3. The setting velocity of the sand is 0.07 ft/s while the clay is considered cohesive with the settling velocity computed internally. The void ratio on deposition is 4.0 for the clay and 0.8 for the sand. The model is used to estimate the concentrations of dissolved plus suspended dredged-material constituents in the water column expressed as a percent of the initial concentration. Water-column bioassays indicated that the LC50 was 30% of the original dredged-material concentration.

B1.8.3.4 Coefficients

Default values were used for all coefficients.

B1.8.3.5 Controls for Execution and Output

The total simulation time is specified as 4 h or 14,400 s, with a 600-s computational time step. Output is specified for depths of 50 and 74 ft, which correspond to near middepth and near bottom, respectively.

B1.8.3.6 Coefficients

Default values were used for all coefficients.

B1.8.3.7 Summary of Output

As can be seen from the output, the jet of material from a bin reaches the bottom after 9.72 s and has a radius of 7.23 ft. The resulting bottom collapse continues as long as the bottom cloud is fed by the continuous discharge of material from the remaining bins. The maximum concentration of suspended plus dissolved constituents of the dredged material after 4 h is 0.0008% of the original concentration, and the maximum concentration outside the disposal site boundary during the 4-h initial-mixing period is 0.0113% of original occurring 80 min after disposal at a depth of 74 ft. Both of these values are below 0.3% (0.01 of the LC50); therefore the discharge is acceptable according to the guidance in Section 11.1.7.

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Figure 3-1. Overview of Tiered Approach to Evaluating Potential Impact of Ocean Disposal of Dredged Material. Sections in which applicable discussions begin in the manual are indicated by the numbers within the parenthesis.

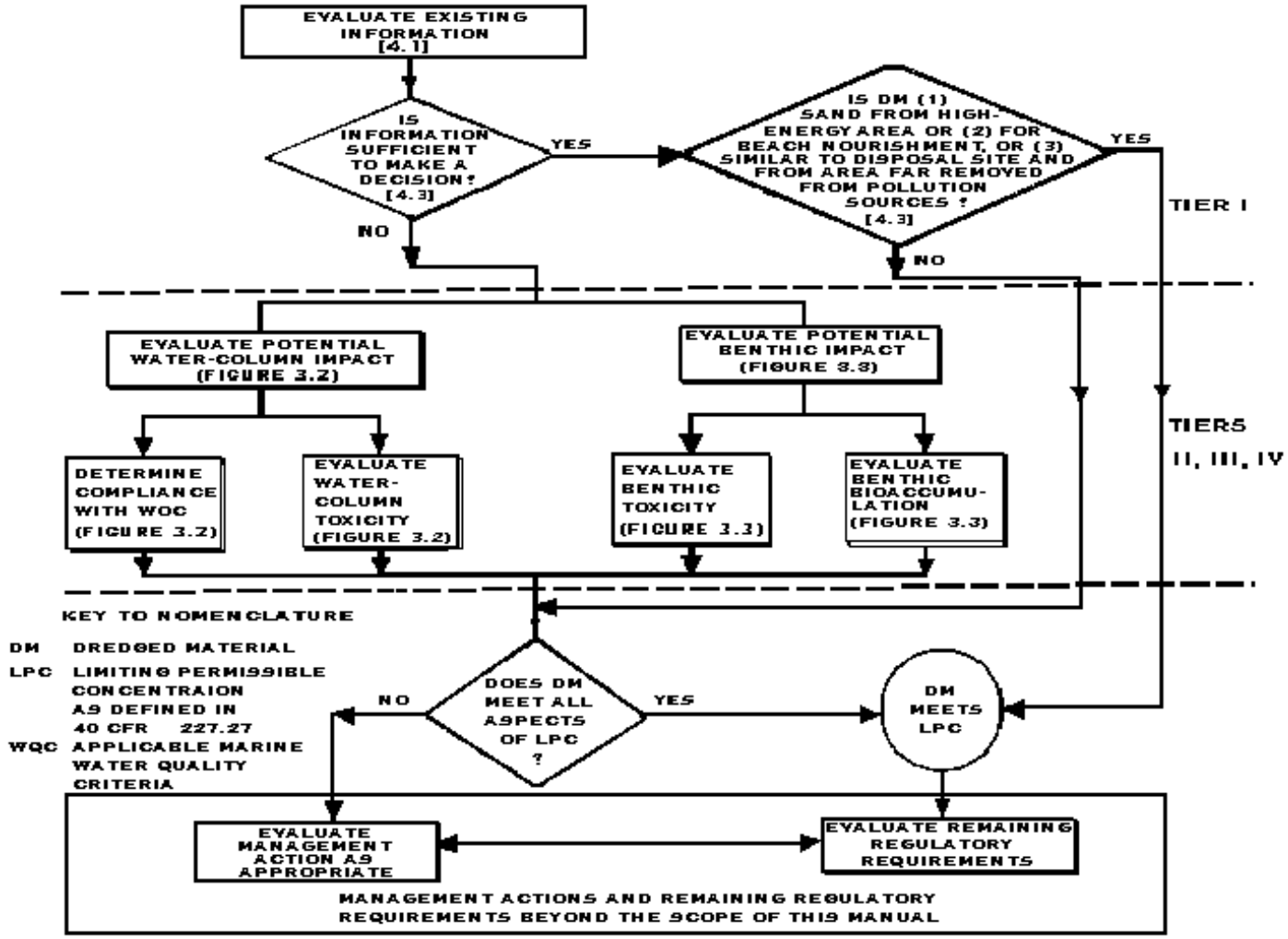

Figure 3-1. Overview of Tiered Approach to Evaluating Potential Impact of Ocean Disposal of Dredged Material. Sections in which applicable discussions begin in the manual are indicated by the numbers within the parenthesis.

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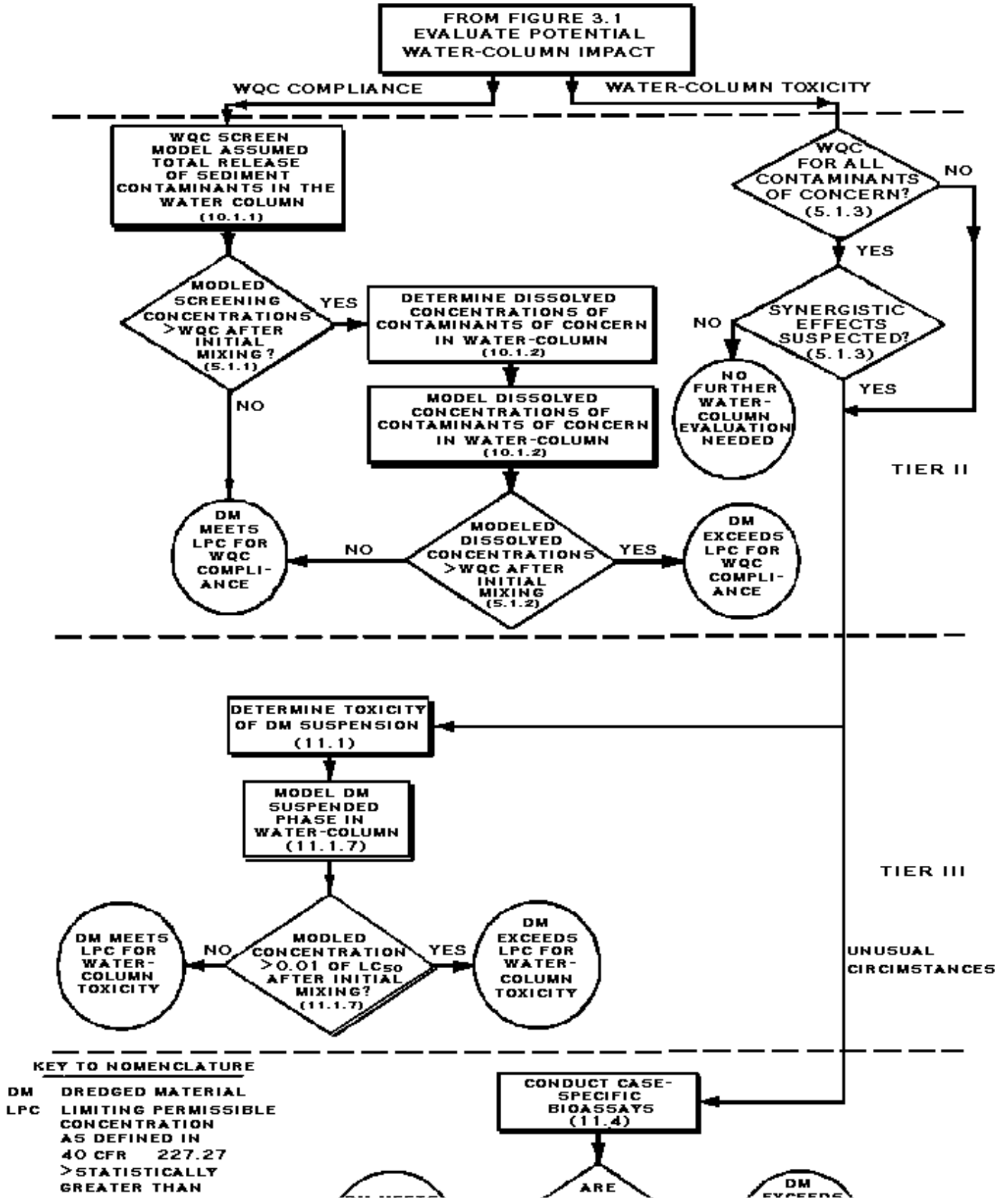
Figure 3-2. Overview of Tiered Approach to Evaluating Potential WATER-COLUMN IMPACT of Dredged Material. Sections in which applicable discussions in the manual are indicated by the numbers within the parentheses.



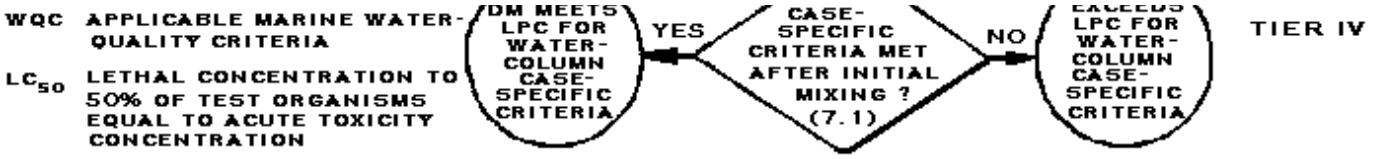


Figure 3-2. Overview of Tiered Approach to Evaluating Potential WATER-COLUMN IMPACT of Dredged Material. Sections in which applicable discussions in the manual are indicated by the numbers within the parentheses.

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Figure 3-3. Tiered Approach to Evaluating Potential BENTHIC IMPACT of Deoposited Dredged material. Sections in which applicable discussions in the manual are indicated by the numbers within the parentheses.

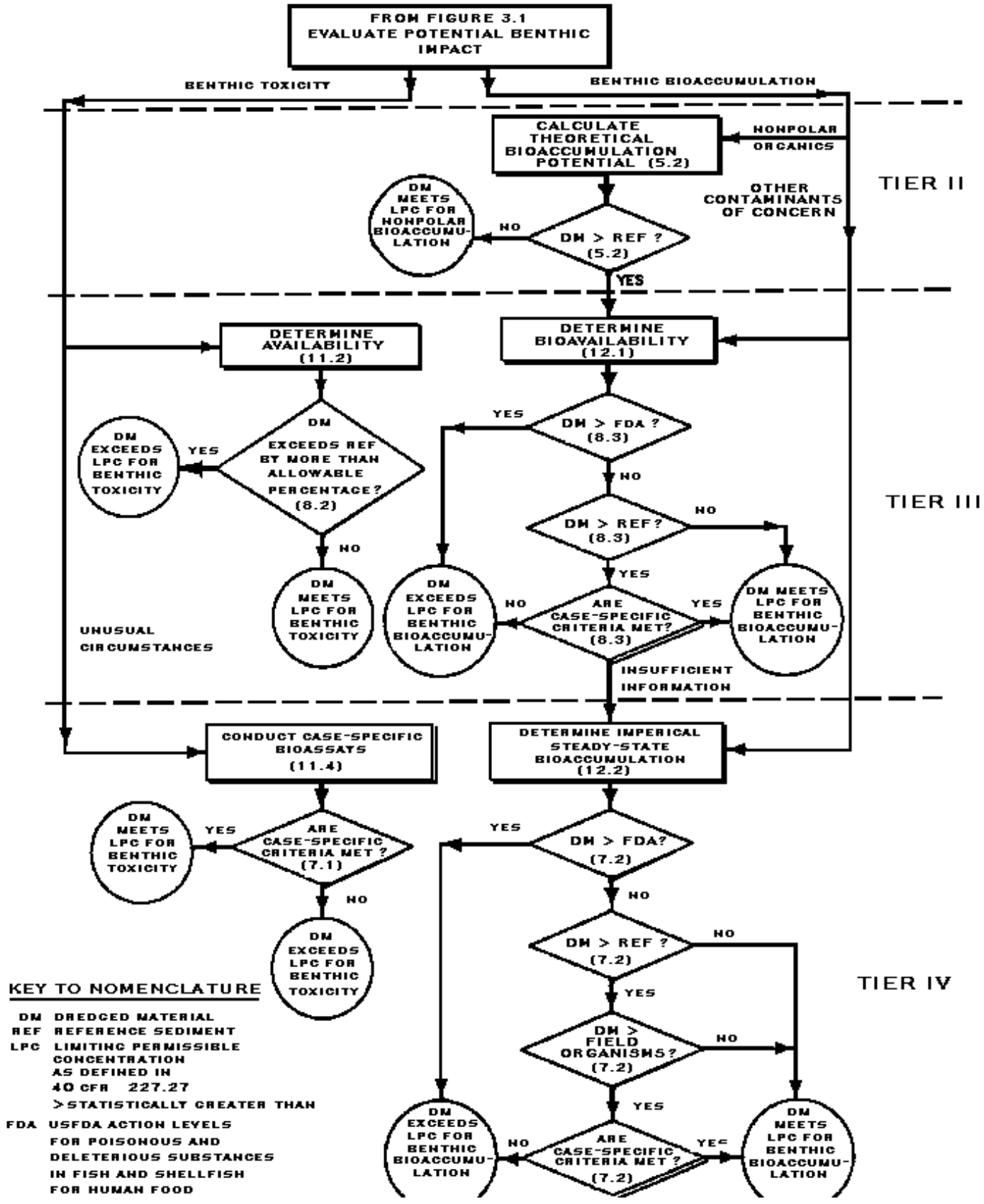

Figure 3-3. Tiered Approach to Evaluating Potential BENTHIC IMPACT of Deoposited Dredged material. Sections in which applicable discussions in the manual are indicated by the numbers within the parentheses.

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This value may be replaced in local guidance if there is a scientific basis for the change. The present EPA/USACE recommendation is that a value of 20% be used for amphipod tests. This recommendation is based on the inherent variability of these tests. If test refinement can reduce this variability, the percentage will be correspondingly reduced to enable more accurate evaluations of the results.

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Table 8.1 **Sample-Collection Requirements. This table contains general guidance on the type of samples that may be required in each tier to conduct dredged-material evaluation tests. Actual sampling requirements are project specific and are determined during the development of the project plan based on the guidance provided in this manual and in regional testing manuals.**

Tests	Water Samples			Sediment Samples		
	Disposal Site	Dredging Site	Control Site	Dredged Material	Reference Sediment	Control Sediment
Tier II						
Water-column						
Screen	●			●		
Elutriate	●	●		●		
Tier II						
Benthic				●	●	
Tier III						
Water-column	●	●	●	●		
Tier III						
Benthic				●	●	●
Tier IV						
Water-column	●	●	●	●		
Tier IV						
Benthic				●	●	●

Table 8-2. Summary of Recommended Procedures for Sample Collection, Preservation, and Storage[a]

Analysis or Test	Collection Method	Amount Required	Container	Preservation Technique	Storage Conditions	Storage Duration[b]
SEDIMENT						
Chemical/Physical Analysis						
Bulk metals	Grab/corer	200 mL	Precleaned pre-weighed poly-styrene jar[c]	Dry ice[c]	≤—20°C[c]	Hg - 30 days Others - 6 months[d]
Bulk organics [PCBs, pesticides, high molecular weight (HMW) hydrocarbons]	Grab/corer	475 mL	Solvent-rinsed glass jar with Teflon lid[c]	Dry ice[c]	≤—20°C[c]/dark[d]	10 days[d]
Particle size	Grab/corer	75 mL	Whirl-pac bag[c]	Dry ice[c]	≤—20°C[c]	Undetermined
TOC	Grab/corer	3 L	Heat treated glass vials with Teflon-lined lids[c]	Dry ice[c]	≤—20°C[c]	Undetermined
Sediment from which elutriate is prepared	Grab/corer	Depends on tests being performed	Glass with Teflon-lined lid	Completely fill & refrigerate	4°C/dark/airtight	Undetermined
Biological Tests						
Dredged material	Grab/corer	12-15 L per sample	Plastic bag or container[e]	Completely fill & refrigerate; sieve	4°C/dark/airtight	2 weeks[f]
Reference sediment	Grab/corer	45-50 L per test	Plastic bag or container[e]	Completely fill & refrigerate; sieve	4°C/dark/airtight	2 weeks[f]
Control sediment	Grab/corer	21-25 L per test	Plastic bag or container[e]	Completely fill & refrigerate; sieve	4°C/dark/airtight	2 weeks[f]
WATER AND ELUTRIATE						
Particulate analysis	Discrete sampler or pump	500-2000 mL	Plastic or glass	Lugols solution & refrigerate	4°C	Undetermined
Metals	Discrete sampler	1 L	Acid-rinsed	pH <2 with HNO_3[d]	4°C□ 2°C[d]	Hg - 2 weeks

[illegible]	[illegible] or pump	[illegible]	[illegible] polyethylene or glass jar[4]	[illegible]	[illegible]	[illegible] Others - 6 months[3]
Total Kjeldahl nitrogen (TKN)	Discrete sampler or pump	100-200 mL	Plastic or glass[3]	H_2SO_4 to pH <2; refrigerate[3]	4°C[3]	24 h[3]
Chemical oxygen demand (COD)	Discrete sampler or pump	200 L	Plastic or glass[3]	H_2SO_4 to pH <2; refrigerate[3]	4°C[3]	7 days[3]
Total organic carbon (TOC)	Discrete sampler or pump	100 mL	Plastic or glass[3]	H_2SO_4 to pH <2; refrigerate[3]	4°C[3]	<48 h[3]
Total inorganic carbon (TIC)	Discrete sampler or pump	100 mL	Plastic or glass[3]	Airtight seal; refrigerate[3]	4°C[3]	6 months[3]
Phenolics	Discrete sampler or pump	1 L	Glass[3]	0.1-1.0 g $CuSO_4$; H_3PO_4 to pH <4; refrigerate[3]	4°C[3]	24 h[3]
Soluble reactive phosphates	Discrete sampler or pump	—	Plastic or glass[3]	Filter; refrigerate[3]	4°C[3]	24 h[3]
Organics	Discrete sampler or pump	4 L	Amber glass bottle[4]	Airtight seal; refrigerate	4° □ 2°C[4]	5 days[4]
Volatile organics	Discrete sampler or pump	80 mL	Glass vial[4]	HCL preservation in airtight completely filled container[4]	4° □ 2°C[4]	5 days[4]
Total phosphorus	Discrete sampler or pump	—	Plastic or glass[3]	Refrigerate	4°C[3]	7 days[3]
Total solids	Discrete sampler or pump	200 mL	Plastic or glass[3]	Refrigerate	4°C[3]	7 days[3]
Volatile solids	Discrete sampler or pump	200 mL	Plastic or glass[3]	Refrigerate	4°C[3]	7 days[3]
Sulfides	Discrete sampler or pump	—	Plastic or glass[3]	2 mL ZnOAc[3]	Ambient[3]	24 h[3]
TISSUE						
Trace metals	Trawl/Teflon-coated grab	30 g	Double Ziploc[4]	Handle w/nonmetallic forceps; plastic gloves; dry ice[4]	≤—20°C[4]	Hg - 28 days Others - 6 months[1]
PCBs and chlorinated pesticides	Trawl/Teflon-coated grab	100 g	Hexane-rinsed double aluminum foil and double	Handle w/hexane-rinsed stainless steel forceps; dry ice[4]	≤—20°C[4]	10 days[1]

			Ziploc[c]			
Volatile organics	Trawl/Teflon-coated grab	50 g	Heat-cleaned aluminum foil and watertight plastic bag[f]	Covered ice chest[e]	—20°C[l]	10 days[l]
PAHs	Trawl/Teflon-coated grab	50 g	Hexane-rinsed double aluminum foil and double Ziploc[c]	Handle w/hexane-rinsed stainless steel forceps; dry ice[c]	≤20°C[c]	10 days[l]
Lipids	Trawl/Teflon-coated grab	50 g	Hexane-rinsed aluminum foil	Handle w/hexane-rinsed stainless steel forceps; quick freeze	20°C	Undetermined

[a] This table contains only a summary of collection, preservation, and storage procedures for samples. The cited references should be consulted for a more detailed description of these procedures.
[b] These are holding times for sediment, water, and tissue. References should be consulted if holding times for sample extracts are desired.
[c] NOAA (1989)
[d] Tetra Tech (1986a)
[e] Prolypropylene should be used if phalate bioaccumulation is of concern.
[f] Two weeks is recommended; up to 6 weeks is acceptable.
[g] EPA (1983)
[h] Plumb (1981)
[i] Tetra Tech (1986b)

Table 9-1. Priority Pollutants and 301(h) Pesticides Listed According to Structural Compound Class

Structural Compound Class	PP* Pollutant	Pollutant
Phenols	65	phenol
	34	2,4-dimethylphenol
Substituted Phenols	21	2,4,6-trichlorophenol
	22	para-chloro-meta-cresol
	24	2-chlorophenol
	31	2,4-dichlorophenol
	57	2-nitrophenol
	58	4-nitrophenol
	59	2,4-dinitrophenol
	60	4,6-dinitro-o-cresol
	64	pentachlorophenol
Organonitrogen	5	benzidine
Compounds	28	3,3'-dichlorobenzidine
	35	2,4-dinitrotoluene
	36	2,6-dinitrotoluene
	37	1,2-diphenylhydrazine
	56	nitrobenzene
	61	*N*-nitrosodimethylamine
	62	*N*-nitrosodiphenylamine
	63	*N*-nitrosodipropylamine
Low Molecular Weight	1	acenaphthene
Polynuclear Aromatic	55	naphthalene
Hydrocarbons (PAH)	77	acenaphthylene
	78	anthracene
	81	phenanthrene
	80	fluorene
High Molecular Weight	39	fluoranthene
Polynuclear Aromatic	72	benzo(*a*)anthracene
Hydrocarbons (PAH)	73	benzo(*a*)pyrene
	74	benzo(*b*)fluoranthene
	75	benzo(*k*)fluoranthene
	76	chrysene
	79	benzo(*g,h*)perylene
	82	dibenzo(*a,h*)anthracene
	83	indeno(1,2,3-*cd*)pyrene

Structural Compound Class	PP*	
Chlorinated Aromatic	8	1,2,4-trichlorobenzene
Hydrocarbons	9	hexachlorobenzene
	20	2-chloronaphthalene
	25	1,2-dichlorobenzene
	26	1,3-dichlorobenzene
	27	1,4-dichlorobenzene
Chlorinate Aliphatic	52	hexachlorobutadiene
Hydrocarbons	12	hexachloroethane
	53	hexachlorocyclopentadiene
Halogenated Ethers	18	bis(2-chloroethyl)ether
	40	4-chlorophenyl ether
	41	4-bromophenyl ether
	42	bis(2-chloroisopropyl)ether
	43	bis(2-chloroethoxy)methane
Phthalates	66	bis(2-ethylhexyl)phthalate
	67	butyl benzyl phthalate
	68	di-*n*-butyl phthalate
	69	di-*n*-octyl phthalate
	70	diethyl phthalate
	71	dimethyl phthalate
Polychlorinated	106	PCB-1242
Biphenyls (PCB)	107	PCB-1254
as Arocolors	108	PCB-1221
	109	PCB-1232
	110	PCB-1248
	111	PCB-1260
	112	PCB-1016
Miscellaneous	129	TCDD (dioxin)
Oxygenated	54	isophorone
Compounds		
Volatile Aromatic	4	benzene
Hydrocarbons	38	ethylbenzene
	86	toluene
Volatile Chlorinated	7	chlorobenzene
Aromatic Hydrocarbons		
Volatile Unsaturated	2	acrolein
Carbonyl Compounds	3	acrylonitrile
Volatile Ethers	19	2-chloroethylvinylether
		bis(chloromethyl)ether (removed)

	83	indeno(1,2,3-cd) pyrene
Pesticides	89	aldrin
	90	dieldrin
	91	chlordane
	92	DDT[b]
	95	endosulfan[c]
	98	endrin
	99	endrin aldehyde
	100	heptachlor
	101	heptachlor epoxide
	102	alpha-hexachlorocyclohexane
	103	beta-hexachlorocyclohexane
	104	delta-hexachlorocyclohexane
	105	gamma-hexachlorocyclohexane
	113	toxaphene
	—	mirex[d]
	—	methoxychlor[d]
	—	parathion[e]
	—	malathion[e]
	—	guthion[e]
	—	demeton[e]
Volatile Halogenated Alkanes	6	tetrachloromethane
	10	1,2-dichloroethane
	11	1,1,1-trichloroethane
	13	1,1-dichloroethane
	14	1,1,2-trichloroethane
	15	1,1,2,2-tetrachloroethane
	16	chloroethane
	23	chloroform
	32	1,2-dichloropropane
	44	dichloromethane
	45	chloromethane
	46	bromomethane
	47	bromoform
	48	dichlorobromoethane
	49	fluorotrichloromethane (removed)
	50	dichlorodifluoromethane (removed)
	51	chlorodibromomethane
Volatile Halogenated Alkenes	29	1,1-dichloroethylene
	30	1,2-*trans*-dichloroethylene
	33	*trans*-1,3-dichloropropene
	33	*cis*-1,3-dichloropropene
	85	tetrachloroethene
	87	trichloroethene
	88	vinyl chloride
Metals	114	antimony
	115	arsenic
	117	beryllium
	118	cadmium
	119	chromium
	120	copper
	122	lead
	123	mercury
	124	nickel
	125	selenium
	126	silver
	127	thallium
	128	zinc
Miscellaneous	121	cyanide
	116	asbestos

[a] PP: priority pollutant designation number
[b] Includes DDT, DDD, and DDE
[c] Includes α-endosulfan, β-endosulfan, and endosulfan sulfate.
[d] Chlorinated 301(h) pesticides that are not on the priority pollutant list.
[e] Organophosphorus 301(h) pesticides that are not on the priority pollutant list.

Table 9.2. Sediment Sample-Size Requirements for Chemical and Physical Analyses

Analytical Parameter Delivered to Laboratory	Sediment Sample Size (g, wet wt)
Organic compounds	250
Metals	100
Miscellaneous	50[a]
Grain size	100
Total organic carbon	50
Total solids/specific gravity	50

[a]Miscellaneous sample size should be increased if auxiliary analytes that cannot be included as part of the organic or metal analyses are added to the target list.

Table 9-3. Polychlorinated Biphenyl (PCB) Congeners Recommended for Quantitation as Potential Contaminants of Concern

PCB Congener[a]	Congener Number[b]		
	Summation[c]	Priority[d]	HighestSecond Priority[e]
			a2,4' diCB8
2,2',5 triCB	18		18
2,4,4' triCB	28		
3,4,4' triCB			37
2,2',3,5' tetraCB	44		44
2,2',4,5' tetraCB			99
2,2',5,5' tetraCB	52		52
2,3',4,4' tetraCB	66		
2,3',4',5 tetraCB			70
2,4,4',5 tetraCB			74
3,3',4,4' tetraCB	77	77	
3,4,4',5 tetraCB			81
2,2',3,4,5' pentaCB		87	
2,2',3,4',5 pentaCB		49	
2,2',4,5,5' pentaCB	101	101	
2,3,3',4,4' pentaCB	105	105	
2,3,4,4',5 pentaCB			114
2,3',4,4',5 pentaCB	118	118	
2,3',4,4',6 pentaCB			119
2',3,4,4',5 pentaCB			123
3,3',4,4',5 pentaCB	126	126	
2',3,3',4,4' hexaCB	128	128	
2,2',3,4,4',5' hexaCB	138	138	
2,2',3,5,5',6 hexaCB			151
2,2',4,4',5,5' hexaCB	153	153	
2,3,3',4,4',5 hexaCB		156	
2,3,3',4,4',5 hexaCB			157
2,3,3',4,4',6 hexaCB		158	
2,3',4,4',5,5' hexaCB			167
2,3',4,4',5',6 hexaCB			168
3,3',4,4',5,5' hexaCB	169	169	
2,2',3,3',4,4',5 heptaCB	170	170	
2,2',3,4,4',5,5' heptaCB	180	180	
2,2',3,4,4',5',6 heptaCB		183	
2,2',3,4,4',6,6' heptaCB		184	
2,2',3,4',5,5',6 heptaCB	187		187
2,3,3',4,4',5,5' heptaCB			189
2,2',3,3',4,4',5,6 octaCB	195		
2,2',3,3',4,5,5',6' octaCB			201
2,2',3,3',4,4',5,5',6 nonaCB	206		
2,2',3,3',4,4',5,5',6,6' decaCB	209		

[a] PCB congeners recommended for quantitation, from dichlorobiphenyl (diCB) through decachlorobiphenyl (decaCB).

[b] Congeners are identified by their International Union of Pure and Applied Chemistry (IUPAC) number, as referenced in Ballschmiter and Zell (1980) and Mullen *et al.* (1984).

[c] These congeners are summed to determine total PCB concentration following the approach in NOAA (1989).

[d] PCB congeners having highest priority for potential environmental importance based on potential for toxicity, frequency of occurrence in environmental samples, and relative abundance in animal tissues (McFarland and Clarke, 1989).

[e] PCB congeners having second priority for potential environmental importance based on potential for toxicity, frequency of occurrence in environmental samples, and relative abundance in animal tissues (McFarland and Clarke, 1989).

Table 9-4. Sources of Marine Reference Materials and Standards

Inorganic Constituents

U.S. Department of Commerce
National Institute for Standards and Technology
Office of Standard Reference Materials
Room B3111 Chemistry Building
Gaithersburg, MD 20899
Telephone: (301) 975-6776

Marine Analytical Chemistry Standards Program
National Research Council of Canada
Division of Chemistry
Montreal Road
Ottawa, Ontario, Canada K1A0R9
Telephone: (613) 993-2359

Organic Constituents

U.S. Department of Commerce
National Institute for Standards and Technology
Office of Standard Reference Materials
Room B3111 Chemistry Building
Gaithersburg, MD 20899
Telephone: (301) 975-6776

Marine Analytical Chemistry Standards Program
National Research Council of Canada
Arlantic Research Laboratory
1411 Oxford Street
Halifax, Nova Scotia, Canada B3H3Z1
Telephone: (902) 426-8280

Table 9-5. Octanol/Water Partition Coefficients (K_{ow}) for Organic Compound Priority Pollutants and 301(h) Pesticides[a]

Pollutant	Octanol/Water Partition Coefficients (log K_{ow})	Pollutant	Octanol/Water Partition Coefficients (log K_{ow})
Di-*n*-octyl phthalate	9.2	Acenaphthylene	4.1
Indeno(1,2,3-*cd*)pyrene	7.7	Butyl benzyl phthalate	4.0
Benzo(*ghi*)perylene	7.0	PCB-1221	4.0
PCB-1260	6.9	Hexachloroethane	3.9
Mirex[b]	6.9	Acenaphthene	3.9
Benzo(*k*)fluoranthene	6.8	α-hexachlorocyclohexane	3.8
Benzo(*b*)fluoranthene	6.6	δ-hexachlorocyclohexane	3.8
PCB-1248	6.1	ß-hexachlorocyclohexane	3.8
2,3,7,8-TCDD (dioxin)	6.1	γ-hexachlorocyclohexane	3.8
Benzo(*a*)pyrene	6.0	Parathion[b]	3.8
Chlordane	6.0	Chlorobenzene	3.8
PCB-1242	6.0	2,4,6-trichlorophenol	3.7
4,4'-DDD	6.0	ß-endosulfan	3.6
Dibenzo(*a,h*)anthracene	6.0	Endosulfan sulfate	3.6
PCB-1016	5.9	α-endosulfan	3.6
4,4'-DDT	5.7	Naphthalene	3.6
4,4'-DDE	5.7	Fluorotrichloromethane[c]	3.5
Benzo(*a*)anthracene	5.6	1,4-dichlorobenzene	3.5
Chrysene	5.6	1,3-dichlorobenzene	3.4
Endrin aldehyde	5.6	1,2-dichlorobenzene	3.4
Fluoranthene	5.5	Toxaphene	3.3
Hexachlorocyclopentadiene	5.5	Ethylbenzene	3.1
Dieldrin	5.5	*N*-nitrosodiphenylamine	3.1
Heptachlor	5.4	*P*-chloro-*m*cresol	3.1
Heptachlor epoxide	5.4	2,4-dichlorophenol	3.1
Hexachlorobenzene	5.2	3,3'-dichlorobenzene	3.0
Di-*n*-butyl phthalate	5.1	Aldrin	3.0
4-Bromophenyl phenyl ether	5.1	1,2-diphenylhydrazine	2.9
Pentachlorophenol	5.0	4-nitrophenol	2.9
4-Chlorophenyl phenyl ether	4.9	Malathion[b]	2.9
Pyrene	4.9	Tetrachloroethene	2.9
2-Chloronaphthalene	4.7	4,6-dinitro-*o*-cresol	2.8
Endrin	4.6	Tetrachloroethene	2.6
PCB-1232	4.5	Bis(2-chloroisopropyl)ether	2.6
Phenanthrene	4.5	1,1,1-trichloroethane	2.5
Fluorene	4.4	Trichloroethene	2.4
Anthracene	4.3	2,4-dimethylphenol	2.4
Methoxychlor[b]	4.3	1,1,2,2-tetrachloroethane	2.4
Hexachlorobutadiene	4.3	Bromoform	2.3
1,2,4-trichlorobenzene	4.2	1,2-dichloropropane	2.3
Bis(2-ethylhexyl)phthalate	4.2	Toluene	2.2
1,1,2-trichloroethane	2.2	Dimethyl phthalate	1.6
Guthion[b]	2.2	Chloroethane	1.5
Dichlorodiflouromethane[c]	2.2	2,4-dinitrophenol	1.5
2-chlorophenol	2.2	1,1-dichloroethylene	1.5
Benzene	2.1	Phenol	1.5
Chlorodibromomethane	2.1	1,2-dichloroethane	1.4
2,4-dinitrotoluene	2.1	Diethyl phthalate	1.4

2,6-dinitrotoluene	2.0	*N*-nitrosodipropylamine	1.3
Trans-1,2-dichloropropene	2.0	Dichloromethane	1.3
Cis-1,3-dichloropropene	2.0	2-chloroethylvinylether	1.3
Demeton[b]	1.9	*Bis*(2-chloroethoxy)methane	1.3
Chloloform	1.9	Acrylonitrile	1.2
Dichlorobromomethane	1.9	*Bis*(2-chloroethyl)ether	1.1
Nitrobenzene	1.9	Bromomethane	1.0
Benzidine	1.8	Acrolein	0.9
1,1-dichloroethane	1.8	Chloromethane	0.9
2-nitrophenol	1.8	Vinyl chloride	0.6
Isophorone	1.7	*N*-nitrosodimethylamine	0.6

[a]Adapted from Tetra Tech (1985).
[b]301(h) pesticides not on the priority pollutant list.
[c]No longer on priority pollutant or 301(h) list.

TABLE 9-6. Bioconcentration Factors (BCF) of Priority Pollutants[a]

Pollutant	Log BCF[b]
Metals	
Methylmercury	4.6
Phenylmercury	4.6
Mercuric acetate	3.5
Copper	3.1
Zinc	2.8
Arsenic	2.5
Cadmium	2.5
Lead	2.2
Chromium IV	2.1
Chromium III	2.1
Mercury	2.0
Nickel	1.7
Thallium	1.2
Antimony	ND
Silver	ND
Selenium	ND
Beryllium	ND
Nonmetals	
Cyanide	ND
Asbestos	ND

[a]Adapted from Tetra Tech (1986b).
[b]ND: No data.

Figure 10-1. Nomograph for Determining Theoretical Bioaccumulation Potential

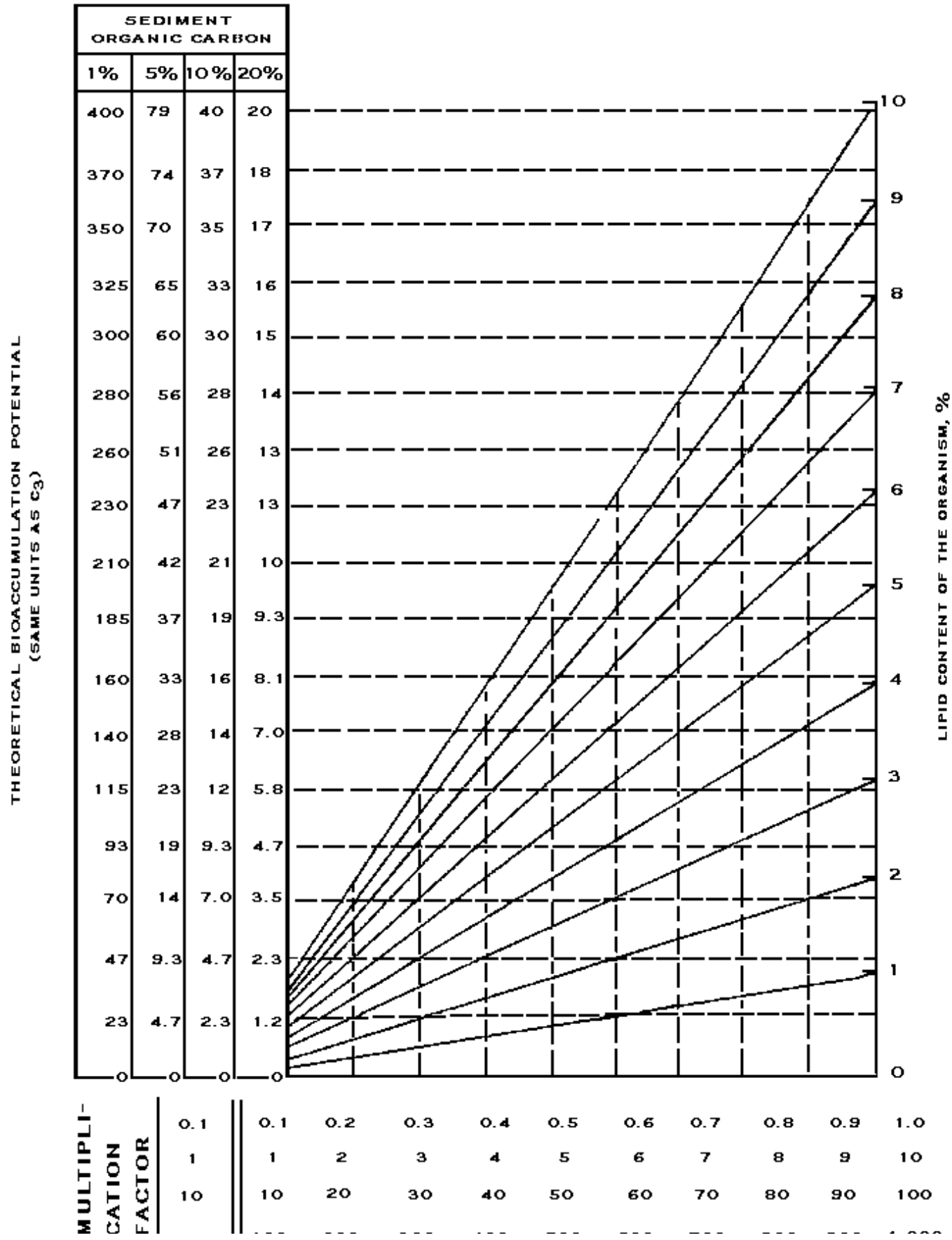

Figure 10-1. Nomograph for Determining Theoretical Bioaccumulation Potential

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Table 11-1. Examples of Appropriate Test Species for Determining Potential Water-Column Impact of Dredged-Material Disposal

Crustaceans

Mysid shrimp, *Mysidopsis* sp.*
Neomysis sp.*
Holmesimysis sp.*

Grass shrimp, *Palaemonetes* sp.

Commercial shrimp, *Penaeus* sp.

Oceanic shrimp, *Pandalus* sp.

Blue crab, *Callinectes sapidus*

Cancer crab, *Cancer* sp.

Fish

Silversides, *Menidia* sp.*

Shiner perch, *Cymatogaster aggregata**

Sheepshead minnow, *Cyprinodon variegatus*

Pinfish, *Lagodon rhomboides*

Spot, *Leiostomus xanthurus*

Sanddab, *Citharicthys stigmaeus*

Grunion, *Leuresthes tenuis*

Dolphinfish, *Coryphaena hippurus*

Zooplankton

Copepods, *Acartia* sp.*

Larvae of
Mussels, *Mytilus edulis**
Oysters, *Crassostrea virginica**
Ostrea sp.*
Sea urchin, *Strongylocentrotus purpuratus*
Lytechinus pictus

Bivalves

Mussel, *Mytilus* sp.

Oyster, *Crassostrea* sp.

Note: Examples are not presented in order of importance; however, the asterisks indicate recommended species.

Table 11-2 Examples of Appropriate Test Species for Determining Potential Benthic Impact of Dredged-Material Disposal

Infaunal Amphipods

Ampelisca sp.*

Rhepoxynius sp.*

Eohaustorius sp.*

Grandiderella japonica

Corophium insidiosum

Burrowing Polychaetes

Neanthes sp.*

Nereis sp.*

Nephthys sp.

Glycera sp.

Arenicola sp.

Abarenicola sp.

Molluscs

Yoldia clam, *Yoldia limatula* sp.

Littleneck clam, *Prototthaca staminea*

Japanese clam, *Tapes japonica*

Crustaceans

Mysid shrimp, *Mysidopsis* sp.
Neomysis sp.
Holmesimysis sp.

Commercial shrimp, *Penaeus* sp.

Grass shrimp, *Palaemonetes* sp.

Sand shrimp, *Crangon* sp.

Ocean shrimp, *Pandalus* sp.

Blue crab, *Callinectes sapidus*

Cancer crab, *Cancer* sp.

Ridge-back prawn, *Sicyonia ingentis*

Fish

Arrow gobi, *Clevelandia ios*

Note: Examples are not presented in order of importance; however, the asterisks indicate recommended species.

Table 13-1. Power Calculations for One-Tailed Tests for Selected Sample Sizes[a] [after Cohen, 1977]

Sample Size	Power(%)[b]
30	99
25	97
20	93
15	86
10	71
9	66
8	62
7	66
6	50
5	43
4	36
3	28
2	20

[a]Where $\alpha = 0.05$ and $\delta/\sigma = 1$.
[b]Power is (1 — ß)100.

Table 13-2. Number of Survivors in a Hypothetical Water-Column Bioassay after 96 h.

Replicate[a]	Concentrations[b]				
	Control[c]	100	50	25	12.5
1	20	6	8	12	17
2	19	7	8	18	17
3	20	9	9	15	18
4	20	5	10	14	16
5	19	8	11	13	18
Totals	98	35	46	72	86

[a]20 organisms per replicate at initiation of the test.
[b]Percent concentrations of dissolved plus suspended dredged-material constituents:

[c]Control : clean seawater.
100% : 1 part suspension and 0 part seawater
50% : 1 part suspension and 1 part seawater
25% : 1 part suspension and 3 parts seawater
12.5% : 1 part suspension and 7 parts seawater

```
<pre>
*****************************************************************
*  This SAS program performs a two-sample t-test on results from  *
*  a 96-hour water column bioassay.  The t-test compares the      *
*  number of surviving organisms in the control (seawater) to the *
*  number of surviving organisms in the 100% concentration.  To   *
*  test for equality of variances between samples, the F' test    *
*  Levene's test are performed.                                   *
*****************************************************************;
options nodate nonumber linesize=80 pagesize=60;

 /* Identify the treatment group codes */
proc format;
  value trtfmt 1='Control'
               2='100%';

 /* Input the bioassay data after the CARDS; statement, listing the */
 /* treatment group code, then the number of survivors in the group */
data susphase;
  input trtmnt num_sviv @@;
  label trtmnt='Treatment Group'
        num_sviv='# of Survivors';
  format trtmnt trtfmt.;
  CARDS;
1 20 1 19 1 20 1 20 1 19
2  6 2  7 2  9 2  5 2  8
;

proc sort data=susphase;
  by trtmnt;

 /* Print out the bioassay data */
PROC PRINT data=susphase label noobs;
  var num_sviv;
  by trtmnt;
  title 'Water Column Bioassay Data Listing';

 /* Perform the two-sample t-test to compare the average number of       */
 /* survivors between the two treatment groups.  The t-statistic will be */
 /* calculated under two scenarios:  when the sample variances are       */
 /* significantly different and when they are not.  The F' test for      */
 /* equality of variance is also performed.                              */
PROC TTEST cochran data=susphase;
  class trtmnt;
  var num_sviv;
  title 'Results of Two-Sample t-test on Water Column Bioassay Data';


 /* Perform Levene's test for equality of sample variances.  This test is */
 /* is not as sensitive to departures from normality as is the F' test.   */
 /* First, calculate the treatment means */
PROC MEANS data=susphase noprint;
  var num_sviv;
  by trtmnt;
  output out=meanout mean=average;

 /* Second, calculate the deviations of responses from their means */
data sustwo;
  merge susphase meanout;
  by trtmnt;
```

```
  deviatns = abs(num_sviv - average);
  label deviatns = 'Absolute Deviation from Average'
        average = 'Group Average';
  keep trtmnt num_sviv average deviatns;

PROC PRINT data=sustwo label noobs;
  var num_sviv average deviatns;
  by trtmnt;
  format average deviatns 4.1;
  title 'Levene''s Test on Water Column Bioassay Data';

 /* Finally, perform the ANOVA on the absolute deviations to perform */
 /* Levene's test                                                    */
PROC GLM data=sustwo;
  class trtmnt;
  model deviatns=trtmnt;
run;
</pre>
```

<pre>
Water Column Bioassay Data Listing

---------- Treatment Group=Control ----------

# of Survivors
20
19
20
20
19

---------- Treatment Group=100% ----------

# of Survivors
6
7
9
5
8

Results of Two-Sample t-test on Water Column Bioassay Data

TTEST PROCEDURE

Variable: NUM_SVIV # of Survivors

TRTMNT	N	Mean	Std Dev	Std Error	Minimum	Maximum
Control	5	19.60000000	0.54772256	0.24494897	19.00000000	20.00000000
100%	5	7.00000000	1.58113883	0.70710678	5.00000000	9.00000000

Variances	T	Method	DF	Prob>|T|
Unequal	16.8375	Satterthwaite	4.9	0.0001
		Cochran	4.0	0.0001
Equal	16.8375		8.0	0.0000

For H0: Variances are equal, F' = 8.33 DF = (4,4) Prob>F' = 0.0640

</pre>

<pre>
Levene's Test on Water Column Bioassay Data

---------- Treatment Group=Control ----------

# of Survivors	Group Average	Absolute Deviation from Average
20	19.6	0.4
19	19.6	0.6
20	19.6	0.4
20	19.6	0.4
19	19.6	0.6

---------- Treatment Group=100% ----------

# of Survivors	Group Average	Absolute Deviation from Average
6	7.0	1.0
7	7.0	0.0
9	7.0	2.0
5	7.0	2.0
8	7.0	1.0

General Linear Models Procedure

Dependent Variable: DEVIATNS Absolute Deviation from Average

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.29600000	1.29600000	3.64	0.0928
Error	8	2.84800000	0.35600000		
Corrected Total	9	4.14400000			

R-Square	C.V.	Root MSE	DEVIATNS Mean
0.312741	71.03064	0.5966574	0.84000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRTMNT	1	1.29600000	1.29600000	3.64	0.0928

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRTMNT	1	1.29600000	1.29600000	3.64	0.0928

</pre>

Table 13-3. Number of Survivors in the Hypothetical Benthic Bioassay

Replicate[a]	Treatments				
			Dredged-Material Locations		
	Reference	Control	Station 1	Station 2	Station 3
1	20	20	17	15	17
2	20	19	16	16	12
3	19	20	18	13	10
4	19	20	17	17	16
5	20	20	15	11	13

[a]20 animals per replicate at initiation of test

```
<pre>
*****************************************************************
*  This SAS program performs a parametric analysis of variance    *
*  (ANOVA) and a nonparametric Kruskal-Wallis test to compare the *
*  average number of surviving organisms in a series of treatment *
*  groups using hypothetical Benthic Bioassay data.  The sample   *
*  treatment averages and standard deviations are also displayed. *
*  For the parametric ANOVA, the program also performs Dunnett's  *
*  test to determine which non-control stations (if any) have     *
*  averages which significantly differ from the reference sample. *
*****************************************************************;
options nodate nonumber linesize=80 pagesize=60;

 /* Identify the treatment group codes */
proc format;
  value trtfmt 1='Reference'
               2='Control'
               3='Statn. 1'
               4='Statn. 2'
               5='Statn. 3';

 /* Input the bioassay data after the CARDS; statement, listing the */
 /* treatment group code, then the number of survivors in the group */
data solphase;
  input trtmnt num_sviv @@;
  prp_sviv = num_sviv/20;            /* Proportion of survivors */
  trn_sviv = arsin(sqrt(prp_sviv));  /* Arcsine transformation of the */
                                     /* proportion                    */
  label trtmnt='Treatment Group'
        num_sviv='# of survivors'
        prp_sviv='Proportion of survivors'
        trn_sviv='Transformed survivorship proportion';
  format trtmnt trtfmt.;
  CARDS;
1 20 1 20 1 19 1 19 1 20
2 20 2 19 2 20 2 20 2 20
3 17 3 16 3 18 3 17 3 15
4 15 4 16 4 13 4 17 4 11
5 17 5 12 5 10 5 16 5 13
;

proc sort data=solphase;
  by trtmnt;

 /* Print out the bioassay data */
PROC PRINT data=solphase label noobs;
  var num_sviv prp_sviv trn_sviv;
  by trtmnt;
  format prp_sviv trn_sviv 5.3;
  title 'Listing of Hypothetical Benthic Bioassay Data';

 /* Obtain the mean number and percentage of survivors per treatment group
*/
PROC MEANS data=solphase noprint;
  var num_sviv prp_sviv trn_sviv;
  by trtmnt;
  output out=meanout mean=m_n m_p m_t
                     std=s_n s_p s_t;

PROC PRINT data=meanout label noobs;
```

```
  var trtmnt m_n s_n m_p s_p m_t s_t;
  label m_n='Avg. # survivors'
        s_n='Std. Dev. for # survivors'
        m_p='Avg. prop. survivors'
        s_p='Std. Dev. for prop. survivors'
        m_t='Avg. transformed prop.'
        s_t='Std. Dev. for transformed prop.';
  format m_n 4.1  m_p 5.3  m_t s_n s_p s_t 5.3;
  title1 'Average and Standard Deviations of Hypothetical Benthic Bioassay';
  title2 'Data, Calculated by Treatment Group';

 /* Perform a parametric one-way ANOVA, with Dunnett's multiple comparisons
*/
 /* test, to determine differences between treatment groups in each of the
*/
 /* responses.  Dunnett's test determines differences between each
*/
 /* treatment group and the reference sample.
*/
PROC GLM order=internal data=solphase;
  class trtmnt;
  model num_sviv trn_sviv = trtmnt;  /* Use transformed proportion response
*/
  means trtmnt/DUNNETTL;
  title
  'Parametric one-way ANOVA on the Hypothetical Benthic Bioassay Data';
  title2 'to determine differences among treatment groups';

 /* Perform a nonparametric one-way ANOVA (Kruskal-Wallis test) on the   */
 /* numbers of survivors to test for differences among treatment groups. */
 /* A nonparametric test is considered due to possible lack of normality */
 /* in the numbers of survivors.                                         */
PROC NPAR1WAY wilcoxon data=solphase;
  class trtmnt;
  var num_sviv;
  title1
  'Nonparametric one-way ANOVA on the Hypothetical Benthic Bioassay Data';
  title2 'to determine differences among treatment groups';
run;
```

Figure 13-4. Example SAS/PC Program for Analyzing Survival Proportion from the Hypothetical
(continued) Benthic Bioassay Data in Table 13-3

</pre>

<pre>
Listing of Hypothetical Benthic Bioassay Data

---------- Treatment Group=Reference ----------

# of survivors	Proportion of survivors	Transformed survivorship proportion
20	1.000	1.571
20	1.000	1.571
19	0.950	1.345
19	0.950	1.345
20	1.000	1.571

---------- Treatment Group=Control ----------

# of survivors	Proportion of survivors	Transformed survivorship proportion
20	1.000	1.571
19	0.950	1.345
20	1.000	1.571
20	1.000	1.571
20	1.000	1.571

---------- Treatment Group=Statn. 1 ----------

# of survivors	Proportion of survivors	Transformed survivorship proportion
17	0.850	1.173
16	0.800	1.107
18	0.900	1.249
17	0.850	1.173
15	0.750	1.047

---------- Treatment Group=Statn. 2 ----------

# of survivors	Proportion of survivors	Transformed survivorship proportion
15	0.750	1.047
16	0.800	1.107
13	0.650	0.938
17	0.850	1.173
11	0.550	0.835

Listing of Hypothetical Benthic Bioassay Data
</pre>

---------- Treatment Group=Statn. 3

# of survivors	Proportion of survivors	Transformed survivorship proportion
17	0.850	1.173
12	0.600	0.886
10	0.500	0.785
16	0.800	1.107
13	0.650	0.938

</pre>

<pre>
</pre>

Average and Standard Deviations of Hypothetical Benthic Bioassay Data, Calculated by Treatment Group

Treatment Group	Avg. # survivors	Std. Dev. for # survivors	Avg. prop. survivors	Std. Dev. for prop. survivors	Avg. transformed prop.	Std. Dev. for transformed prop.
Reference	19.6	0.548	0.980	0.027	1.481	0.124
Control	19.8	0.447	0.990	0.022	1.526	0.101
Statn. 1	16.6	1.140	0.830	0.057	1.150	0.076
Statn. 2	14.4	2.408	0.720	0.120	1.020	0.135
Statn. 3	13.6	2.881	0.680	0.144	0.978	0.160

<pre>
</pre>

<pre>
Parametric one-way ANOVA on the Hypothetical Benthic Bioassay Data
to determine differences among treatment groups

General Linear Models Procedure

Dependent Variable: NUM_SVIV # of survivors

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	164.40000000	41.10000000	12.92	0.0001
Error	20	63.60000000	3.18000000		
Corrected Total	24	228.00000000			

R-Square	C.V.	Root MSE	NUM_SVIV Mean
0.721053	10.61462	1.7832555	16.80000000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRTMNT	4	164.40000000	41.10000000	12.92	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRTMNT	4	164.40000000	41.10000000	12.92	0.0001

General Linear Models Procedure

Dependent Variable: TRN_SVIV Transformed survivorship proportion

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.32120960	0.33030240	22.06	0.0001
Error	20	0.29948815	0.01497441		
Corrected Total	24	1.62069775			

R-Square	C.V.	Root MSE	TRN_SVIV Mean
0.815210	9.941941	0.1223700	1.23084575

Source	DF	Type I SS	Mean Square	F Value	Pr > F

TRTMNT 4 1.32120960 0.33030240 22.06 0.0001

Source DF Type III SS Mean Square F Value Pr > F

TRTMNT 4 1.32120960 0.33030240 22.06 0.0001
</pre>

<pre>
Parametric one-way ANOVA on the Hypothetical Benthic Bioassay Data
to determine differences among treatment groups

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: NUM_SVIV

NOTE: This tests controls the type I experimentwise error for
comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 20 MSE= 3.18
Critical Value of Dunnett's T= 2.304
Minimum Significant Difference= 2.599

Comparisons significant at the 0.05 level are indicated by '***'.

TRTMNT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
Control - Reference	-2.399	0.200	2.799	
Statn. 1 - Reference	-5.599	-3.000	-0.401	***
Statn. 2 - Reference	-7.799	-5.200	-2.601	***
Statn. 3 - Reference	-8.599	-6.000	-3.401	***

Parametric one-way ANOVA on the Hypothetical Benthic Bioassay Data
to determine differences among treatment groups

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: TRN_SVIV

NOTE: This tests controls the type I experimentwise error for
comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 20 MSE= 0.014974
Critical Value of Dunnett's T= 2.304
Minimum Significant Difference= 0.1783

Comparisons significant at the 0.05 level are indicated by '***'.

TRTMNT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
Control - Reference	-0.1332	0.0451	0.2234	
Statn. 1 - Reference	-0.5090	-0.3307	-0.1523	***
Statn. 2 - Reference	-0.6388	-0.4605	-0.2821	***
Statn. 3 - Reference	-0.6810	-0.5027	-0.3244	***
</pre>

<pre>
Nonparametric one-way ANOVA on the Hypothetical Benthic Bioassay Data
to determine differences among treatment groups

N P A R 1 W A Y P R O C E D U R E

Wilcoxon Scores (Rank Sums) for Variable NUM_SVIV
Classified by Variable TRTMNT

TRTMNT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Referenc	5	100.000000	65.0	14.5028733	20.0000000
Control	5	105.000000	65.0	14.5028733	21.0000000
Statn. 1	5	55.500000	65.0	14.5028733	11.1000000
Statn. 2	5	34.500000	65.0	14.5028733	6.9000000
Statn. 3	5	30.000000	65.0	14.5028733	6.0000000

Average Scores were used for Ties

Kruskal-Wallis Test (Chi-Square Approximation)
CHISQ= 19.286 DF= 4 Prob > CHISQ= 0.0007
</pre>

Table 13-4. Results from a Hypothetical Single-Time Point Bioaccumulation Test, Showing Average Contaminant Concentrations (μg/g dry weight) in Tissues of Animals Exposed to Different Treatments

Replicate[a]					
			Dredged-Material Samples		
	Reference	Control	1	2	3
1	0.06	0.04	0.16	0.24	0.13
2	0.05	0.03	0.19	0.10	0.05
3	0.05	0.09	0.18	0.13	0.17
4	0.08	0.04	0.22	0.18	0.08
5	0.09	0.05	0.31	0.30	0.22
n	5		5	5	5
Mean	0.066		0.212	0.190	0.130
Standard error	0.008		0.026	0.036	0.030
Upper 95%, one-sided confidence limit	0.083				
Lower 95%, one-sided confidence limit			0.156	0.113	0.065

[a]20 animals per replicate

Table 13-5. Selected Values of the Two-Tailed *t* Distribution

Degrees of Freedom	Value of *t* Distribution[a]
1	6.314
2	2.920
3	2.353
4	2.132
5	2.015
6	1.943
7	1.895
8	1.860
9	1.833
10	1.812

[a]Two-tailed probability: 0.10
One-tailed probability: 0.05

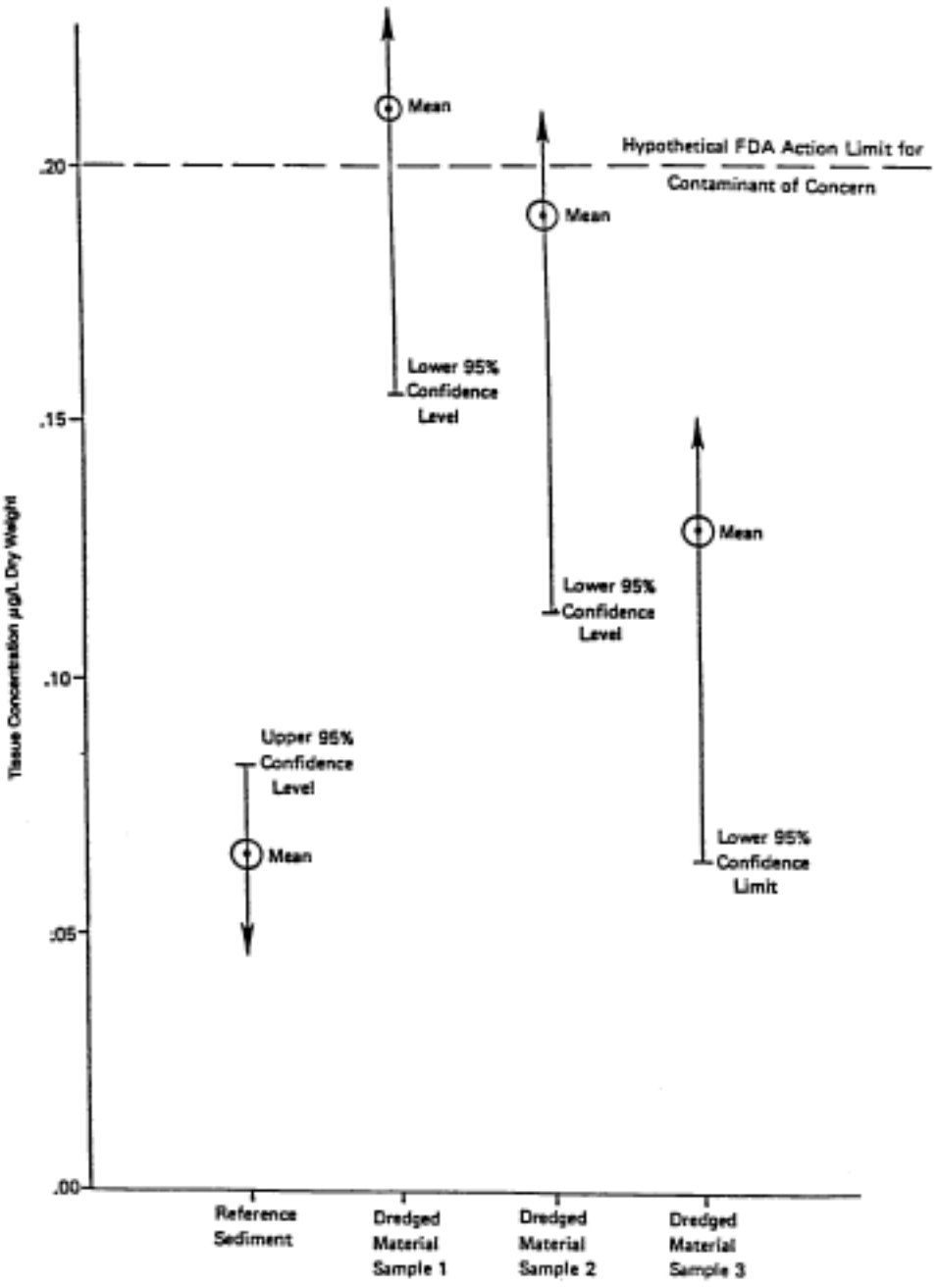
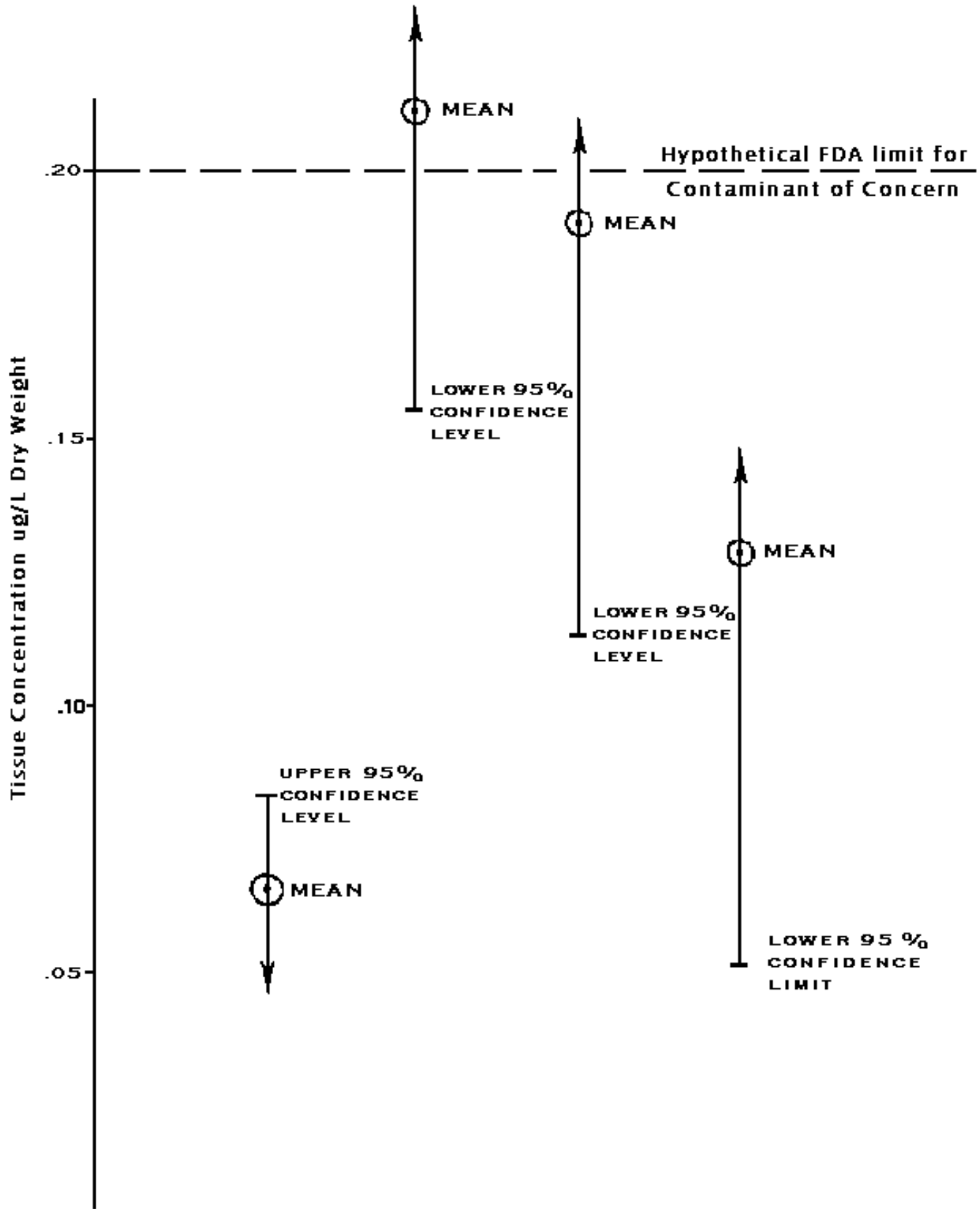


Figure 13-10. Mean Tissue Concentration with 95% One-Sided Confidence Intervals Calculated on Hypothetical Single-Time Point Bioaccumulation Data Given in Table 13-4

Figure 13-10. Mean Tissue Concentration with 95% One-Sided Confidence Intervals Calculated on Single-Time Point Bioaccumulation Data Given in Table 13-4.



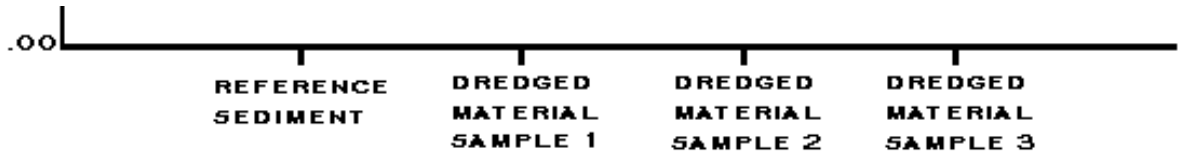


Figure 13-10. Mean Tissue Concentration with 95% One-Sided Confidence Intervals Calculated on Single-Time Point Bioaccumulation Data Given in Table 13-4.

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Table 13-6. Average Tissue Concentration Resulting from a Hypothetical 28-Day Time-Series Bioaccumulation Test, Showing Different Contaminant Concentrations in Tissues of Animals Exposed to Different Treatments[a]

Day	Replicate	Reference	Dredged Material Samples		
			A	B	C
2	1	0.054	0.159	0.869	0.745
2	2	0.163	0.292	0.726	1.703
2	3	0.391	0.428	0.394	2.045
2	4	0.734	0.558	1.232	1.855
2	5	0.634	0.256	0.977	.135
4	1	0.441	0.516	0.838	1.316
4	2	0.797	0.158	0.633	0.930
4	3	0.203	0.743	0.452	2.141
4	4	0.564	0.324	0.728	1.150
4	5	0.018	0.126	1.314	1.621
7	1	0.687	0.881	1.246	1.583
7	2	0.177	0.317	0.816	2.715
7	3	0.862	0.270	0.897	1.016
7	4	0.413	0.562	1.639	2.221
7	5	0.029	0.095	0.688	2.134
10	1	0.037	0.278	1.767	1.578
10	2	0.549	0.485	1.272	2.268
10	3	0.884	0.051	1.003	1.756
10	4	0.787	0.909	1.158	2.899
10	5	0.294	0.718	1.415	0.890
18	1	0.856	0.904	1.631	2.822
18	2	0.598	1.300	1.877	2.607
18	3	0.016	0.671	1.487	3.414
18	4	0.806	0.234	1.216	1.319
18	5	0.119	0.337	1.280	1.866
28	1	0.514	0.172	1.178	1.295
28	2	0.839	1.049	1.721	2.964
28	3	0.793	0.476	1.366	2.109
28	4	0.099	0.712	1.513	2.820
28	5	0.226	1.245	1.843	3.325
Mean sediment concentration		0.4	54.0	33.0	44.0

[a] Total contaminant concentration in micrograms per gram dry weight

- Total contaminant concentration in micrograms per gram dry weight.

Reference Sediment Statistics

Steady-state mean tissue concentration: 0.473 g/g.

Steady-state upper 95%, one-sided confidence limit: 0.590.

Hypothetical FDA action level: 2 µg/g

```
<pre>
*********************************************************************
*  This SAS program performs a nonlinear regression analysis to fit *
*  a simple kinetic model on hypothetical 28-day bioaccumulation    *
*  laboratory test data.  This analysis determines if there are     *
*  differences between steady state bioaccumulation in organisms    *
*  exposed to dredged material and in organisms exposed to          *
*  reference sediment.  This program also calculates one-sided 95%  *
*  confidence limits from the two-sided limits calculated by PROC   *
*  NLIN.  The program assumes a sample size of five.                *
*********************************************************************;
options nodate nonumber linesize=80 pagesize=60;

 /* Identify the station codes */
proc format;
  value $trtfmt 'R'='Reference'
                'A'='Station A'
                'B'='Station B'
                'C'='Station C';

 /* Input the bioaccumulation data after the CARDS; statement, listing the
*/
 /* station code, the day of measurement, and the tissue concentration.
*/
data bioaccum;
  input trtmnt $ t_days c1-c5 @@;
  array cs{5} c1-c5;

 /* Input the mean sediment concentration in the following SELECT statement
*/
  select (trtmnt);
     when ('R') conc_sed = 0.45;  /* Reference sediment concentration */
     when ('A') conc_sed = 4.0;   /* Station A sediment concentration */
     when ('B') conc_sed = 33.;   /* Station B sediment concentration */
     when ('C') conc_sed = 44.;   /* Station C sediment concentration */
     otherwise;
  end;

  /* Output one line per measurement */
  do rep=1 to 5;
     conc_tis = cs{rep};
     output;
  end;

  keep trtmnt t_days conc_sed rep conc_tis;
  format trtmnt $trtfmt.;
  label trtmnt='Treatment Level'
        t_days='  Time (days) '
        conc_sed='  Sediment Concentration '
        rep='Replicate Number'
        conc_tis='Tissue Concentration';

  CARDS;
R  2 0.054 0.163 0.391 0.734 0.634    R  4 0.441 0.797 0.203 0.564 0.018
R  7 0.687 0.177 0.862 0.413 0.029    R 10 0.037 0.549 0.884 0.787 0.294
R 18 0.856 0.598 0.016 0.806 0.119    R 28 0.514 0.839 0.793 0.099 0.226

A  2 0.159 0.292 0.428 0.558 0.256    A  4 0.516 0.158 0.743 0.324 0.126
A  7 0.881 0.317 0.270 0.562 0.095    A 10 0.278 0.485 0.051 0.909 0.718
A 18 0.904 1.300 0.671 0.234 0.337    A 28 0.172 1.049 0.476 0.712 1.245
```

```
B  2 0.869 0.726 0.394 1.232 0.977    B  4 0.838 0.633 0.452 0.728 1.314
B  7 1.246 0.816 0.897 1.639 0.688    B 10 1.767 1.272 1.003 1.158 1.415
B 18 1.631 1.877 1.487 1.216 1.280    B 28 1.178 1.721 1.366 1.513 1.843
C  2 0.745 1.703 2.045 1.855 1.135    C  4 1.316 0.930 2.141 1.150 1.621
C  7 1.583 2.715 1.016 2.221 2.134    C 10 1.578 2.268 1.756 2.899 0.890
C 18 2.822 2.607 3.414 1.319 1.866    C 28 1.295 2.964 2.109 2.820 3.325
;
proc sort data=bioaccum;
  by trtmnt conc_sed t_days rep;

 /* Print the input data */
PROC PRINT data=bioaccum label noobs;
  var rep conc_tis;
  by trtmnt conc_sed t_days;
  title 'Listing of 28-Day Bioaccumulation Data';

 /* Fit the simple kinetic model on the data */
data bioaccum;
  set bioaccum;
  by trtmnt;
  output;
  if (last.trtmnt) then do;
                        t_days = 999;
                        rep = 1;
                        conc_tis = .;
                        output;
                        end;

PROC NLIN data=bioaccum method=marquardt;
  by trtmnt;
  parameters k1=0.1 k2=0.5;
  kicks = k1*conc_sed/k2;
  exp_term = exp(-k2*t_days);
  model conc_tis = kicks*(1-exp_term);
  der.k1 = (conc_sed/k2) * (1-exp_term);
  der.k2 = kicks * (-1/k2 + exp_term/k2 + t_days*exp_term);
  output out=results
         p=pred_ct l95m=lo_95_2s u95m=up_95_2s;
  title 'Fitting of Kinetic Model to the Bioaccumulation Data';

 /* Calculate the 95% one-sided confidence limits based on the */
 /* two-sided limits calculated by PROC NLIN.                  */

proc means data=results noprint;
  var conc_tis;
  by trtmnt;
  output out=nreps n=n;

data results2;
  merge results nreps;
  by trtmnt;
  if (rep = 1);
  df = n - 2;
  t_05 = tinv(0.975,df);
  t_10 = tinv(0.95,df);
  lo_95_1s = pred_ct - (up_95_2s - pred_ct)*t_10/t_05;
  up_95_1s = pred_ct + (up_95_2s - pred_ct)*t_10/t_05;
  label pred_ct='Predicted Concentration'
        lo_95_1s='Lower 95% Conf. Bound on the Concentration'
        up_95_1s='Upper 95% Conf. Bound on the Concentration';
```

```
proc sort data=results2;
  by trtmnt conc_sed t_days;

PROC PRINT data=results2 label noobs;
  var t_days pred_ct lo_95_1s up_95_1s;
  by trtmnt conc_sed;
  format pred_ct lo_95_1s up_95_1s 6.4;
  title 'Listing of Predicted Tissue Concentrations and One-Sided 95%';
  title2 'Confidence Intervals, Based on the Fitted Kinetic Model';
run;
</pre>
```

<pre>
Listing of 28-Day Bioaccumulation Data

----- Treatment Level=Station A Sediment Concentration=4 Time (days)=2 -----

Replicate Number	Tissue Concentration
1	0.159
2	0.292
3	0.428
4	0.558
5	0.256

----- Treatment Level=Station A Sediment Concentration=4 Time (days)=4 -----

Replicate Number	Tissue Concentration
1	0.516
2	0.158
3	0.743
4	0.324
5	0.126

----- Treatment Level=Station A Sediment Concentration=4 Time (days)=7 -----

Replicate Number	Tissue Concentration
1	0.881
2	0.317
3	0.270
4	0.562
5	0.095

---- Treatment Level=Station A Sediment Concentration=4 Time (days)=10 -----

Replicate Number	Tissue Concentration
1	0.278
2	0.485
3	0.051
4	0.909
5	0.718

---- Treatment Level=Station A Sediment Concentration=4 Time (days)=18 -----

Replicate Number	Tissue Concentration
1	0.904
2	1.300
3	0.671

Replicate Number	Tissue Concentration
4	0.234
5	0.337

---- Treatment Level=Station A Sediment Concentration=4 Time (days)=28

Replicate Number	Tissue Concentration
1	0.172
2	1.049
3	0.476
4	0.712
5	1.245

---- Treatment Level=Station B Sediment Concentration=33 Time (days)=2

Replicate Number	Tissue Concentration
1	0.869
2	0.726
3	0.394
4	1.232
5	0.977

---- Treatment Level=Station B Sediment Concentration=33 Time (days)=4

Replicate Number	Tissue Concentration
1	0.838
2	0.633
3	0.452
4	0.728
5	1.314

---- Treatment Level=Station B Sediment Concentration=33 Time (days)=7

Replicate Number	Tissue Concentration
1	1.246
2	0.816
3	0.897
4	1.639
5	0.688

---- Treatment Level=Station B Sediment Concentration=33 Time (days)=10

Replicate Number	Tissue Concentration
1	1.767
2	1.272
3	1.003

Replicate Number	Tissue Concentration
4	1.158
5	1.415

---- Treatment Level=Station B Sediment Concentration=33 Time (days)=18

Replicate Number	Tissue Concentration
1	1.631
2	1.877
3	1.487
4	1.216
5	1.280

---- Treatment Level=Station B Sediment Concentration=33 Time (days)=28

Replicate Number	Tissue Concentration
1	1.178
2	1.721
3	1.366
4	1.513
5	1.843

---- Treatment Level=Station C Sediment Concentration=44 Time (days)=2

Replicate Number	Tissue Concentration
1	0.745
2	1.703
3	2.045
4	1.855
5	1.135

---- Treatment Level=Station C Sediment Concentration=44 Time (days)=4

Replicate Number	Tissue Concentration
1	1.316
2	0.930
3	2.141
4	1.150
5	1.621

---- Treatment Level=Station C Sediment Concentration=44 Time (days)=7

Replicate Number	Tissue Concentration
1	1.583
2	2.715

Replicate Number	Tissue Concentration
3	1.016
4	2.221
5	2.134

---- Treatment Level=Station C Sediment Concentration=44 Time (days)=10

Replicate Number	Tissue Concentration
1	1.578
2	2.268
3	1.756
4	2.899
5	0.890

--- Treatment Level=Station C Sediment Concentration=44 Time (days)=18

Replicate Number	Tissue Concentration
1	2.822
2	2.607
3	3.414
4	1.319
5	1.866

--- Treatment Level=Station C Sediment Concentration=44 Time (days)=28

Replicate Number	Tissue Concentration
1	1.295
2	2.964
3	2.109
4	2.820
5	3.325

--- Treatment Level=Reference Sediment Concentration=0.45 Time (days)=2

Replicate Number	Tissue Concentration
1	0.054
2	0.163
3	0.391
4	0.734
5	0.634

--- Treatment Level=Reference Sediment Concentration=0.45 Time (days)=4

Replicate Number	Tissue Concentration
1	0.441
2	0.797

Replicate Number	Tissue Concentration
3	0.203
4	0.564
5	0.018

--- Treatment Level=Reference Sediment Concentration=0.45 Time (days)=7 ----

Replicate Number	Tissue Concentration
1	0.687
2	0.177
3	0.862
4	0.413
5	0.029

--- Treatment Level=Reference Sediment Concentration=0.45 Time (days)=10 ---

Replicate Number	Tissue Concentration
1	0.037
2	0.549
3	0.884
4	0.787
5	0.294

--- Treatment Level=Reference Sediment Concentration=0.45 Time (days)=18 ---

Replicate Number	Tissue Concentration
1	0.856
2	0.598
3	0.016
4	0.806
5	0.119

--- Treatment Level=Reference Sediment Concentration=0.45 Time (days)=28 ---

Replicate Number	Tissue Concentration
1	0.514
2	0.839
3	0.793
4	0.099
5	0.226

</pre>

<pre>
Fitting of Kinetic Model to the Bioaccumulation Data

-------------------------- Treatment Level=Reference

Non-Linear Least Squares Iterative Phase
Dependent Variable CONC_TIS Method: Marquardt

Iter	K1	K2	Sum of Squares
0	0.100000	0.500000	6.887855
1	0.685462	1.283176	4.167862
2	0.974848	0.687322	3.452842
3	0.785682	0.730668	2.755431
4	0.802025	0.761427	2.753115
5	0.811932	0.772154	2.753084
6	0.815045	0.775362	2.753082
7	0.815940	0.776284	2.753082
8	0.816195	0.776546	2.753082

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable CONC_TIS

Source	DF	Sum of Squares	Mean Square
Regression	2	6.1793341786	3.0896670893
Residual	28	2.7530818214	0.0983243508
Uncorrected Total	30	8.9324160000	
(Corrected Total)	29	2.7815808000	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
K1	0.8161949523	0.72854762039	-.67615585015	2.3085457547
K2	0.7765458839	0.74248899959	-.74436232210	2.2974540900

Asymptotic Correlation Matrix

Corr	K1	K2
K1	1	0.9899643378
K2	0.9899643378	1

</pre>

<pre>
Fitting of Kinetic Model to the Bioaccumulation Data

-------------------------- Treatment Level=Station A

Non-Linear Least Squares Iterative Phase
Dependent Variable CONC_TIS Method: Marquardt

Iter	K1	K2	Sum of Squares
0	0.100000	0.500000	4.511244
1	0.032072	0.283014	3.513831
2	0.032303	0.157206	3.041152
3	0.029106	0.164033	2.856415
4	0.029372	0.167118	2.856061
5	0.029488	0.168038	2.856044
6	0.029522	0.168305	2.856043
7	0.029532	0.168382	2.856043
8	0.029534	0.168404	2.856043

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable CONC_TIS

Source	DF	Sum of Squares	Mean Square
Regression	2	8.249353153	4.124676577
Residual	28	2.856042847	0.102001530
Uncorrected Total	30	11.105396000	
(Corrected Total)	29	3.377693467	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
K1	0.0295344074	0.01095794141	0.00708825264	0.05198056222
K2	0.1684037645	0.08228376939	-.00014561487	0.33695314391

Asymptotic Correlation Matrix

Corr	K1	K2
K1	1	0.9540322074
K2	0.9540322074	1

</pre>

<pre>
Fitting of Kinetic Model to the Bioaccumulation Data

-------------------------- Treatment Level=Station B

Non-Linear Least Squares Iterative Phase
Dependent Variable CONC_TIS Method: Marquardt

Iter	K1	K2	Sum of Squares
0	0.100000	0.500000	717.141922
1	0.010591	0.448632	10.506473
2	0.013544	0.250922	4.997893
3	0.010636	0.240108	2.892513
4	0.010558	0.235466	2.888916
5	0.010522	0.234465	2.888869
6	0.010514	0.234235	2.888867
7	0.010512	0.234181	2.888867
8	0.010512	0.234169	2.888867

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable CONC_TIS

Source	DF	Sum of Squares	Mean Square
Regression	2	43.269707380	21.634853690
Residual	28	2.888866620	0.103173808
Uncorrected Total	30	46.158574000	
(Corrected Total)	29	4.913541467	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
K1	0.0105115591	0.00190839085	0.00660242738	0.01442069084
K2	0.2341690260	0.05242599994	0.12678004972	0.34155800218

Asymptotic Correlation Matrix

Corr	K1	K2
K1	1	0.9631505062
K2	0.9631505062	1

</pre>

<pre>
Fitting of Kinetic Model to the Bioaccumulation Data

-------------------------- Treatment Level=Station C

Non-Linear Least Squares Iterative Phase
Dependent Variable CONC_TIS Method: Marquardt

Iter	K1	K2	Sum of Squares
0	0.100000	0.500000	1140.757812
1	0.018864	0.469373	17.310419
2	0.018651	0.346647	13.626377
3	0.017109	0.332666	13.307998
4	0.016865	0.326231	13.305115
5	0.016748	0.323514	13.304649
6	0.016698	0.322342	13.304561
7	0.016676	0.321833	13.304544
8	0.016667	0.321611	13.304541
9	0.016662	0.321515	13.304541
10	0.016661	0.321472	13.304541

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable CONC_TIS

Source	DF	Sum of Squares	Mean Square
Regression	2	116.05813143	58.02906572
Residual	28	13.30454057	0.47516216
Uncorrected Total	30	129.36267200	
(Corrected Total)	29	16.29165320	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
K1	0.0166606579	0.00451591707	0.00741029143	0.02591102431
K2	0.3214724020	0.10238980337	0.11173799211	0.53120681186

Asymptotic Correlation Matrix

Corr	K1	K2
K1	1	0.9717375672
K2	0.9717375672	1

</pre>

<pre>
Listing of Predicted Tissue Concentrations and One-Sided 95%
Confidence Intervals, Based on the Fitted Kinetic Model

---------- Treatment Level=Station A Sediment Concentration=4

Time (days)	Predicted Concentration	Lower 95% Conf. Bound on the Concentration	Upper 95% Conf. Bound on the Concentration
2	0.2006	0.0990	0.3022
4	0.3438	0.2060	0.4817
7	0.4857	0.3497	0.6217
10	0.5713	0.4516	0.6910
18	0.6677	0.5244	0.8109
28	0.6952	0.5070	0.8834
999	0.7015	0.4931	0.9099

---------- Treatment Level=Station B Sediment Concentration=33

Time (days)	Predicted Concentration	Lower 95% Conf. Bound on the Concentration	Upper 95% Conf. Bound on the Concentration
2	0.5540	0.4262	0.6817
4	0.9008	0.7490	1.0525
7	1.1937	1.0663	1.3211
10	1.3389	1.2266	1.4512
18	1.4594	1.3105	1.6084
28	1.4792	1.3088	1.6496
999	1.4813	1.3070	1.6557

---------- Treatment Level=Station C Sediment Concentration=44

Time (days)	Predicted Concentration	Lower 95% Conf. Bound on the Concentration	Upper 95% Conf. Bound on the Concentration
2	1.0815	0.7440	1.4189
4	1.6500	1.3136	1.9864
7	2.0401	1.7958	2.2843
10	2.1888	1.9462	2.4313
18	2.2734	1.9606	2.5861
28	2.2801	1.9534	2.6067
999	2.2803	1.9528	2.6079

---------- Treatment Level=Reference Sediment Concentration=0.45

Time (days)	Predicted Concentration	Lower 95% Conf. Bound on the Concentration	Upper 95% Conf. Bound on the Concentration

2	0.3729	0.1511	0.5947
4	0.4518	0.3421	0.5615
7	0.4709	0.3623	0.5795
10	0.4728	0.3570	0.5885
18	0.4730	0.3559	0.5900
28	0.4730	0.3559	0.5900
999	0.4730	0.3559	0.5900

</pre>

Figure 13-18. Nonlinear Regression Analysis Lines with 95% One-Sided Confidence Bounds on Bioaccumulation Data

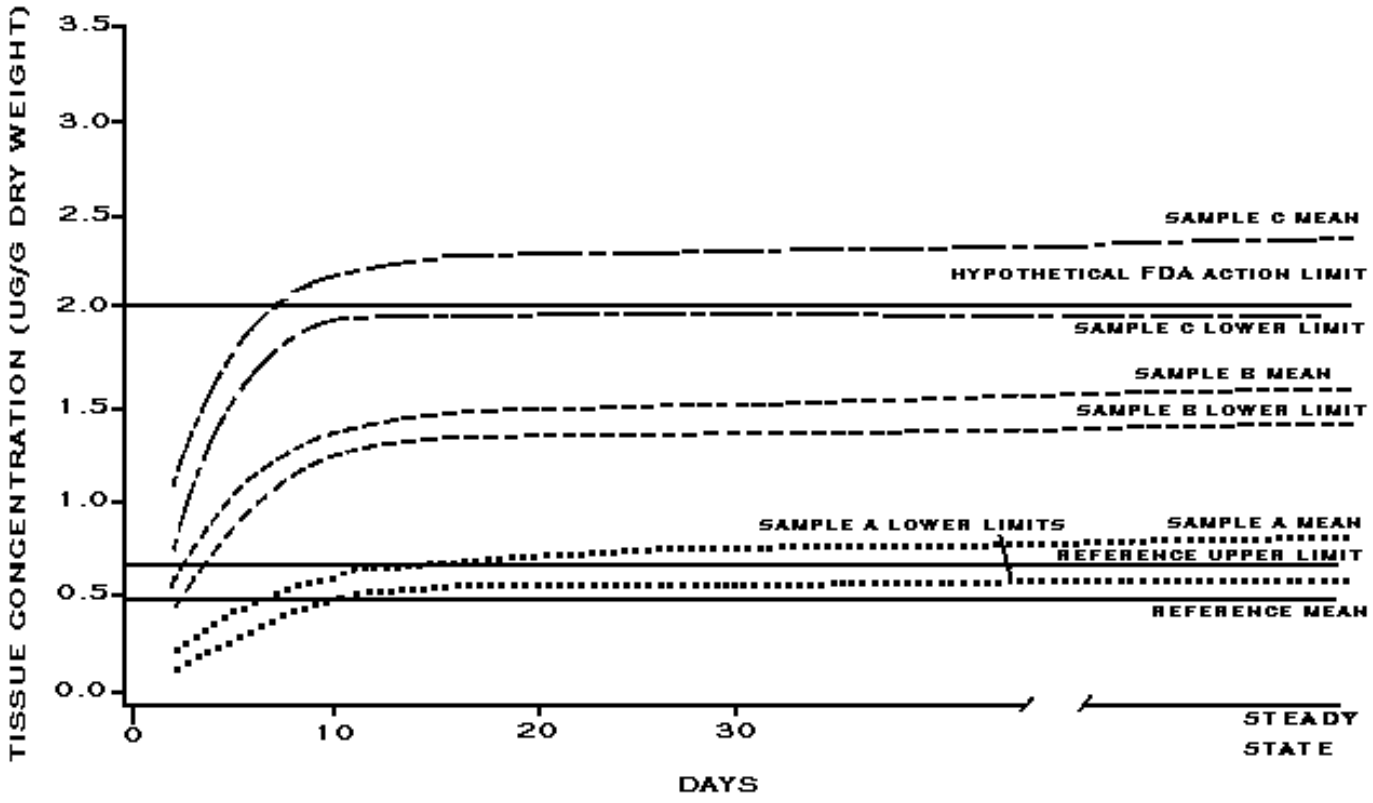

Figure 13-18. Nonlinear Regression Analysis Lines with 95% One-Sided Confidence Bounds on Bioaccumulation Data

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TABLE B-1. MODEL INPUT PARAMETERS

Parameter	Models*	Units	Option*
Disposal Site Descriptions			
Descriptive title	I,C,H		
Gridpoints (left to right)	I,C,H		
Gridpoints (top to bottom)	I,C,H		
Distance between gridpoints	I,C,H	ft	
Constant water depth	I,C,H	ft	C
Gridpoints depths	I,C,H	ft	V
Points in density profile	I,C,H		
Depth of density point	I,C,H	ft	
Density at profile point	I,C,H	g/c^3	
Bottom slope in *x* direction	I,H	deg	
Bottom slope in *z* direction	I,H	deg	
Site boundary grid locations	I,C,H		
Disposal Operation Descriptions			
Volume of material in barge	I	yd^3	
Discharge flow rate	C,H	ft^3/s	
Radius of discharge	C,H	ft	
Discharge depth	C,H	ft	
Angle of discharge	C	deg	
Vessel course	C	deg	
Vessel speed	C	ft/s	
Barge velocity in *x* direction	I	ft/s	
Barge velocity in *z* direction	I	ft/s	
Barge length	I	ft	
Barge width	I	ft	
Postdisposal depth	I	ft	
Bottom depression length in *x* direction	I,H	ft	Optional
Bottom depression length in *z* direction	I,H	ft	Optional
Bottom depression depth	I,H	ft	Optional
X coordinate of disposal operation	I,C,H	ft	
Z coordinate of disposal operation	I,C,H	ft	
Disposal duration	I,C,H	s	
Time from start of tidal cycle	I,C,H	s	
Number of hopper bins opening together	H		
Distance between bins	H	ft	
Disposal Site Velocity Descriptions			
Type of velocity profile	I,C,H		
Tidal cycle time of velocity if constant profile not used	I,C,H	s	V
Vertically averaged velocity in *x* direction at gridpoints	I,C,H	ft/s	V
Vertically averaged velocity in *z* direction at gridpoints	I,C,H	ft/s	V
Velocity in *x* direction at upper point	I,C,H	ft/s	C
Depth of upper point for *x* direction velocity	I,C,H	ft	C
Velocity in *x* direction at lower point	I,C,H	ft/s	C
Depth of lower point for *x* direction velocity	I,C,H	ft	C
Velocity in *z* direction at upper point	I,C,H	ft/s	C
Depth of upper point for *z* direction velocity	I,C,H	ft	C

Velocity in z direction at lower point	I,C,H	ft/s	C
Depth of lower point for z direction velocity	I,C,H	ft	C
Material Descriptions			
Water density at dredging site	I,C,H	g/c^3	
Number of solid fractions	I,C,H		
Solid-fraction descriptions	I,C,H		
Solid-fraction specific gravity	I,C,H		
Solid-fraction volumetric concentration	I,C,H	ft^3/ft^3	
Solid-fraction settling velocity	I,C,H	ft/s	
Solid-fraction deposited void ratio	I,C,H		
Moisture content of material in barge as multiple of liquid limit	I		Cohesive
Bulk density of dredged material	I,C,H	g/c^3	
Dissolved contaminant concentration	I,C,H	mg/L	Optional
Background dissolved contaminant concentration	I,C,H	mg/L	Optional
Sediment contaminant concentration	I,C,H	mg/kg	Optional
Contaminant water-quality criterion	I,C,H	mg/L	Optional
0.01 of the acutely toxic concentration (LC_{50})	I,C,H	%	Optional
Model coefficient			
Settling coefficient	I,C,H		
Apparent mass coefficient	I,C,H		
Drag coefficient	I,C,H		
Form drag for collapsing cloud	I,C,H		
Skin friction for collapsing cloud	I,C,H		
Drag for an ellipsoidal wedge	I,C,H		
Drag for a plate	I,C,H		
Friction between cloud and bottom	I,C,H		
Horizontal diffusion coefficient	I,C,H		
Cloud/ambient density gradient ratio	I,C,H		
Turbulent thermal entrainment	I,H		
Entrainment in collapse	I,H		
Jet entrainment	H,C		
Thermal entrainment	H,C		
Entrainment by convection in collapse	C		
Entrainment due collapse of element	C		
Input, Output, and Execution Descriptions			
Processes to simulate	I,C,H		
Type of computations to perform for initial mixing	I,C,H		
Number of depths for initial-mixing calculations	I,C,H		
Depths for initial-mixing calculations	I,C,H	ft	
Duration of simulation	I,C,H	s	
Time steps for initial-mixing calculations	I,C,H		
Convective descent output option	I,C,H		
Collapse phase output option	I,C,H		
Number of print times for initial-mixing output	I,C,H		

[1]The use of a parameter in the DIFID, DIFCD, and DIFHD models is indicated in the table by either I, C, or H, respectively.

- The use of a parameter for the constant-depth option or variable-depth option is indicated in the table by either C or V, respectively. Other optional uses for parameters are so indicated.