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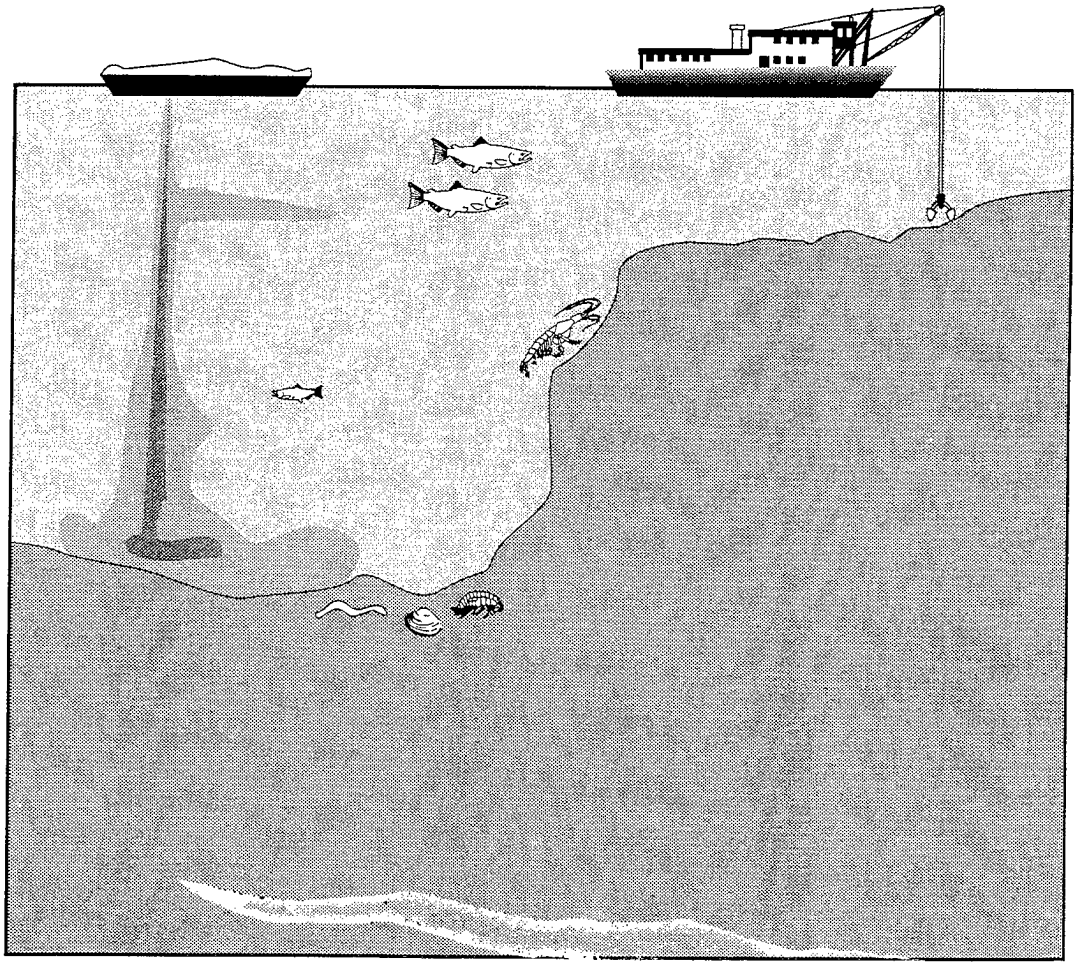
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Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. - Testing Manual

Inland Testing Manual

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United States
Army Corps of Engineers

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Implementation Memorandum for
"Evaluation of Dredged Material Proposed for Discharge
in Waters of the U.S. - Testing Manual"
Inland Testing Manual

Summary

Attached is a copy of the U.S. Army Corps of Engineers (Corps) and Environmental Protection Agency (EPA) document "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual." This document is commonly referred to as the "Inland Testing Manual" (ITM). The purpose of the ITM is to provide guidance regarding technical protocols under Section 404 of the Clean Water Act (CWA) for evaluating proposed discharges of dredged material associated with navigational dredging projects into waters of the United States. This memorandum provides background information on the ITM, describes its scope and applicability, and outlines a schedule for its implementation. In accordance with that schedule, the ITM will be phased in over the next 18 months.

Background

In 1992, a workgroup of Corps and EPA researchers, technical specialists, and policymakers convened to develop an updated technical document to evaluate proposed discharges of dredged material (associated with navigational dredging projects) into waters of the United States. The workgroup effort was designed to develop a manual that would replace existing national guidance for CWA Section 404 waters ("Ecological Evaluation of Proposed Discharge of Dredged or Fill Material into Navigable Waters," 1976; commonly referred to as the "Gold Book") and serve as the counterpart to the recently revised guidance for ocean disposal of dredged material regulated under the Marine, Protection, Research, and Sanctuaries Act, or MPRSA ("Evaluation of Dredged Material Proposed for Ocean Disposal--Testing Manual," 1991; commonly referred to as the "Green Book" or "Ocean Testing Manual").

In 1994, a draft of the document was distributed for public comment. A Notice was published in the Federal Register announcing the availability of the draft document for review and copies were sent to Federal and State agencies, port authorities, environmental organizations, and other interested parties. Public meetings were also held in 1994 to discuss the document in Boston, MA, Arlington, VA, Atlanta, GA, San Jose, CA, Seattle, WA, Chicago, IL, St. Louis, MO, and Houston, TX. Altogether, about 2,000 copies of the draft testing manual were distributed. Comments received through the public review process, including those from EPA's Science Advisory Board, were used to shape the final document. Many individuals and groups provided useful and insightful recommendations throughout the ITM development process and

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their time and effort is greatly appreciated. Modifications were made in the final ITM, where appropriate, based on these comments. A copy of the comments, and EPA's response, is available for review at EPA's Water Docket (202-260-3027).

The attached ITM incorporates a number of scientific advances since issuance of the 1976 manual, including new laboratory techniques, test species, procedures, detection limits, and evaluation protocols that represent the current state of knowledge for dredged material testing and evaluation. The document's tiered approach to testing is designed to provide the information needed to determine the potential for contaminant-related impacts of proposed discharges without necessitating unnecessary testing and evaluation. The ITM is also structured to allow the incorporation of improved methods and techniques as the state of the science advances.

Applicability

The ITM applies to the evaluation of proposed discharges of dredged material associated with navigational dredging projects into waters of the United States, where disposal is proposed for open water. The technical methods described are not generally suitable for, and are not intended to apply to, dredged material resulting from activities such as landclearing and ditching, even though these discharges may require authorization under CWA Section 404. Where non-navigational dredging and subsequent discharge activities are of essentially the same character as navigational dredging and disposal in open water, the ITM may be applied (e.g., open water discharges of dredged material excavated from a soft-bottom flood control channel or reservoir). The technical methods described are not generally suitable for, and are not intended to apply to, the evaluation of dredged material discharges to uplands or to waters of the U.S. that are not typically inundated, i.e., discharge locations in uplands or waters of the U.S. that do not reflect conditions similar to open water disposal, such as discharges to levees or seasonal wetlands[1]. In addition, technical methods described are not generally suitable for, and are not intended to apply to, the evaluation of fill material. Where the ITM methods are not applicable, or there is any question about applicability, potential applicants are advised to contact their local Corps or EPA offices for further information.

The ITM is also intended to be applied in a manner that effectively considers the potential for the proposed discharge of dredged material to cause adverse impacts on the aquatic ecosystem. The level of review required under the Section 404(b)(1) Guidelines will vary with the nature of potential impacts associated with a particular project. In particular, smaller dredged material disposal projects with minor potential impacts will typically demand less characterization of the material in question, and as a result less comprehensive evaluation, as long as sufficient information is collected and documented to reach a determination regarding compliance with the Section 404(b)(1) Guidelines. The Guidelines recognize that "Although all requirements...must be met, the compliance evaluation procedures will vary to reflect the seriousness of the potential for adverse impacts on the aquatic ecosystems posed by specific dredged or fill material discharge activities" [40 CFR 230.10] and that "it generally is not intended or expected that extensive testing, evaluation or analysis will be needed to make

[1]Note, however, that an appendix in the ITM does address those circumstances in which dredged material is proposed for discharge in contained upland disposal sites where there will be return flows into waters of the U.S.

findings of compliance" in cases with little "potential for significant degradation of the aquatic environment" [40 CFR 230.6]. The Guidelines also provide regional flexibility for the preparation of implementation guidance, including testing provisions, as long as this guidance does not modify the basic application, meaning, or intent of the Guidelines [40 CFR 230.2 and 230.61]. The manual provides the best available guidance regarding how dredged material should be tested. It is intended solely as guidance and therefore does not impose any legally binding requirements on Federal agencies, States, or the regulated community.

Implementation Schedule

This February 1998 Inland Testing Manual replaces the 1976 "Ecological Evaluation of Proposed Discharge of Dredged or Fill Material into Navigable Waters," manual in its entirety for implementation of the testing requirements of the December 24, 1980 CWA Section 404 (b)(1) guidelines. The process outlined below will ensure orderly implementation of the ITM and provide adequate opportunity for CWA Section 404 permit applicants to consult with the agencies regarding their specific circumstances.

- Testing or evaluations conducted to support permits or Corps approvals issued prior to the date of this memo remain valid until permit/approval expiration. Where such permits/approvals are renewable on a periodic basis (e.g., every five years), evaluations remain valid until the end of the existing period (e.g., the end of the current five year period).

- Permits or Corps approvals issued or renewed on or after the date of this memo should be supported by testing/evaluation under the procedures of the ITM, unless all of the following three conditions are met:

    - Sampling and analysis plans are submitted to the Corps and EPA on or before August 1, 1998; AND

    - Sampling and analysis plans are approved by the Corps and EPA on or before October 1, 1998; AND

    - Completed test results are submitted to the Corps and EPA for review on or before March 1, 1999.[2]

      Permits or Corps approvals "grandfathered" in accordance with the above three conditions should thereafter be subject to testing and evaluation under the ITM at the time of their next issuance/renewal.

- In order to enable applicants to develop sampling and analysis plans beginning August 1, 1998, EPA Regions and Corps Districts should issue by June 1, 1998, at a minimum, those general regional provisions necessary for the submittal of those plans, e.g., regional

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[2]Where tests have been conducted according to the approved sampling and analysis plan, any additional testing or analysis required of the applicant should be consistent with the protocols used to formulate the original sampling and analysis plan.

contaminants of concern, regional test species. Corps Districts and EPA Regions should complete additional local agreements and regional manuals, as deemed necessary to supplement the ITM to reflect regional circumstances, as quickly as possible but no later than July 1, 1999. These documents will be published for public review and comment (and furnished to Corps and EPA headquarters) prior to final issuance.

- Corps Districts will begin immediately to utilize public notification mechanisms under the CWA, MPRSA, and Rivers and Harbors Act (RHA) to inform the public and potential applicants about the ITM and the above schedule. Those Corps Districts and EPA Regions which have been utilizing procedures or protocols in the draft ITM, or regional manuals developed in accordance with those procedures and protocols, should review those procedures for consistency with the final ITM in accordance with the above implementation schedule. In addition, applicants that choose to proceed under the final ITM prior to the phase-in times are encouraged to work with the appropriate Corps District and EPA Region to do so.

Reference Sediment Rule

As of the publication date of this manual, testing requirements in the Section 404(b)(1) Guidelines regarding the point of comparison for evaluating proposed discharges of dredged material are being updated to provide for comparison to a "reference sediment" as opposed to sediment from the disposal site. Because discharges at a disposal site could impact the point of comparison for future discharges at that site, adoption of a reference sediment that is unimpacted by previous discharges of dredged material will result in a more scientifically sound evaluation of potential individual and cumulative contaminant-related impacts. This change to the Guidelines was proposed in the Federal Register in January 1995, public comments have been received, and a final rule Notice is being prepared. Our agencies expect that the final rule will be published prior to the first phase-in date for ITM implementation, August 1, 1998, and as a result the reference sediment approach will be implemented in the ITM. Revised text for the ITM will be added as necessary to reflect the final rule. Any questions regarding the reference sediment rule and its applicability to the ITM should be directed to the EPA or Corps offices below.

Inland Testing Manual Format and Future Revisions

As technical advances in dredged material testing and assessment are made, the ITM may be revised. When the Corps and EPA agree, at the national level, with new procedures for testing dredged material, older sections to this loose leaf format will be replaced. Interested parties will be notified in advance of dredged material testing changes, as appropriate, through the Corps public notification process. Comments from interested parties will be considered and appropriate phase-in of new testing protocols will give applicants ample opportunity to become familiar with the revised procedures. All changes will be sequentially numbered and posted on the Internet web addresses for the ITM.

The ITM is available on Worldwide Web at the Corps Dredging Operations Technical Support home page at: http://www.wes.army.mil/el/dots/, or at EPA web site

http://www.epa.gov/OST/pubs/ITM.html. Paper copies of the ITM can be obtained by contacting:

Inland Testing Manual Mailing List
c/o Mr. Thomas Patin
U.S. Army Corps of Engineers
Waterways Experiment Station
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

Questions regarding the technical procedures and implementation of the ITM should be directed to your local Corps and EPA offices. Policy and programmatic matters may be raised to the Operations, Construction, and Readiness Division of Corps Headquarters (202-761-0199), or to the Wetlands Division of EPA Headquarters (202-260-7791).

JOHN H. ZIRSCHKY
Acting Assistant Secretary of the
Army (Civil Works)

ROBERT PERCIASEPE
Assistant Administrator for Water
U.S. Environmental Protection Agency

EVALUATION OF DREDGED MATERIAL PROPOSED FOR DISCHARGE IN WATERS OF THE U.S. - TESTING MANUAL

(INLAND TESTING MANUAL)

Prepared by

ENVIRONMENTAL PROTECTION AGENCY
Office of Water
Office of Science and Technology
Washington, D.C.

and

DEPARTMENT OF THE ARMY
United States Army Corps of Engineers
Operations, Construction, and Readiness Division
Washington, D.C.

February 1998

The testing protocols set out in the Inland Testing Manual are intended solely as guidance for use in conducting testing of dredged material to assess the potential for contaminant-related impacts associated with dredged material disposal into open water. The Manual does not alter the statutory and regulatory framework for permitting decisions under section 404 of the CWA. Under that framework, testing is conducted in order to assist the permitting authority in making factual determinations regarding the effect of the discharge on the aquatic ecosystem, and in determining whether the discharge will comply with the 404(b)(1) Guidelines. See 40 C.F.R. 230.10 and 230.11. The current regulations provide for testing under certain circumstances, and this Manual provides suggested protocols to follow once it has been decided that testing is appropriate. The Guidelines provide flexibility to the permitting authority to decide, based upon the facts of a particular case, whether testing is warranted.

The Manual is intended solely as guidance. The Manual is not intended, nor can it be relied upon, to create any rights or obligations enforceable by any party. The Manual provides the best available technical guidance regarding how dredged material should be tested. While it is generally anticipated that the Agencies will follow the procedures in this Manual, Agency decision-makers retain the discretion to adopt approaches on a case-by-case basis that differ from the guidance in the Manual where determined to be appropriate. The document does not, and is not intended to, impose any legally-binding requirements on Federal agencies, States, or the regulated community.

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PREFACE

The "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual", commonly referred to as the Inland Testing Manual represents a major effort by the U.S. Army Corps of Engineers (USACE) and the Environmental Protection Agency (EPA) to establish procedures applicable to the evaluation of potential contaminant-related environmental impacts associated with the discharge of dredged material in inland waters, near coastal waters, and surrounding environs (that is, all waters other than the ocean and the territorial seas, regulated pursuant to Section 404, CWA). This manual is consistent, to the maximum extent practicable, with the procedures established for ocean waters (i.e., the "Green Book" entitled "Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual" - EPA/USACE, 1991). The USACE and EPA have statutory and regulatory responsibilities with regard to the management of dredged material discharge activities in inland and near coastal waters. The USACE is responsible for regulating non-Federal dredging and dredged material discharge activities through a permit program, and for conducting Federal dredging and dredged material discharge activities in conjunction with its Civil Works Program. EPA is responsible for establishing, in conjunction with the USACE, guidelines pertaining to the evaluation of these activities, and performing oversight actions. Specifically, Section 404 of the Federal Water Pollution Control Act of 1972 (FWPCA), Public Law 92-500, as amended by the Clean Water Act of 1977 (CWA), Public Law 95-217, requires, among other things, that the discharge of dredged or fill material into waters of the U.S. be permitted by the USACE. The USACE also conducts Civil Works dredging and dredged material discharge activities in accordance with Section 404. Section 404 further requires that discharge sites be specified though the application of the Section 404(b)(1) Guidelines (Guidelines) developed by EPA in conjunction with the USACE. Section 404 requires that the "guidelines shall be based upon criteria comparable to the criteria applicable to the territorial seas, contiguous zone, and the ocean". Thus, a clear connection for comparable testing for ocean, inland and near coastal waters was established as early as 1972.

The Guidelines, which impart other requirements in addition to those associated with contaminant-related impacts, are published at 40 CFR 230. This manual provides testing procedures applicable to determining the potential for contaminant-related environmental impacts associated with the discharge of dredged material. Dredged material evaluated under the procedures described in this manual must also satisfy all other applicable requirements of 40 CFR 230-232, 33 CFR 320-330, and 33 CFR 335-338 in order to comply with the Guidelines and to be authorized for discharge.

This manual, which is designed to allow for regional flexibility in implementation and application including development of regional manuals and documentation, will be periodically revised and updated as warranted by advances in regulatory practice and technical understanding. This manual replaces the May 1976 proposed testing protocol, "Ecological Evaluation of Proposed Discharge of Dredged or Fill Material Into Navigable Waters", which will no longer be applicable. The 1976 protocol was developed in response to a requirement in the Federal Register notice of the Guidelines, Vol. 40, No. 173, Friday, 5 September 1975. That notice states the "EPA in conjunction with the Corps of Engineers will publish a procedures manual that will cover summary and description of tests, definitions, sample collection and preservation, procedures, calculations and references." In December 1980, the Guidelines were revised and finalized in the Federal Register Vol. 45, No. 249. The present joint effort by EPA and USACE contains up-to-date testing procedures to implement the Guidelines at Sections 230.60 and 230.61, and is

intended to bring compatibility and a comparable level of environmental protection for dredged material testing in ocean, inland and near coastal waters.

This manual is one of a series of guidance documents jointly developed by EPA and the USACE pertaining to dredged material disposal. This series includes a document entitled "Evaluating Environmental Effects of Dredged Material Management Alternatives - A Technical Framework" (Framework Document - USACE/EPA, 1992). The Framework Document articulates those factors to be considered in identifying the environmental effects of dredged material management alternatives on a continuum of discharge sites from uplands to the oceans (management alternatives include open water, confined and beneficial use situations) that meet the substantive and procedural requirements of the National Environmental Policy Act (NEPA), the CWA and the Marine Protection, Research, and Sanctuaries Act (MPRSA). The Green Book (EPA/USACE, 1991) is included in the series. Application of the testing guidance in this manual in addition to guidance provided in the Framework Document and the Green Book will allow for consistency in decision making with respect to technical considerations, across statutory boundaries and the continuum of dredged material discharge options.

The contributions made by many individuals from both agencies are gratefully acknowledged. The first and second drafts of the manual were completed by the Environmental Laboratory (EL) of the USACE Waterways Experiment Station (WES): Thomas Wright, primary author; Michael Palermo, author of Appendix B; Paul Schroeder, Michael Palermo, Robert Randall and Billy Johnson, authors of Appendix C. Succeeding drafts were completed by an EPA/USACE Workgroup established by EPA's Office of Science and Technology (OST) within the Office of Water (OW). Mike Kravitz of OST was the Work Assignment Manager. Appendix D was written by Dennis Brandon and Joan Clarke (WES) and Michael Paine (EVS Consultants). Appendix F was written by Gary Ankley (EPA). Appendix G was written by Sandra Salazar and Peter Chapman (EVS Consultants). Henry Lee and Bruce Boese (EPA) contributed valuable information pertaining to sediment bioaccumulation testing. Carie Schaffer and Robert Johnson (Tetra Tech, Inc.) provided computer support for internet and electronic versions of the document, respectively.

The Workgroup was comprised of individuals from headquarters, field offices and research laboratories of both agencies with scientific and/or programmatic experience related to dredged material discharge activities.

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Review of this manual was conducted by EPA through OW [OST and the Office of Wetlands, Oceans and Watersheds (OWOW)] and by USACE through the Office of the Chief of Engineers (Regulatory Branch, Dredging and Navigation Branch, Office of Environmental Policy) and EL of WES. In addition, the results of the EPA's Science Advisory Board (SAB, 1992) review of the 1991 Green Book were considered in detail, where applicable, during development of this manual. The results of EPA's SAB (1994) review of the draft Inland Testing Manual were considered during its finalization. Regional issues which have National relevance were provided by EPA Region and USACE Division and District staff, and were incorporated into the appropriate sections of this document. This manual provides comprehensive testing guidance from a national perspective. Within the framework of this document, EPA Regions and USACE Districts and Divisions will develop region-specific guidance and/or procedures, as necessary (e.g., region-specific test species), to provide sufficient information to make informed dredged material discharge decisions.

This manual should be cited as follows:

EPA/USACE. 1998. Evaluation of dredged material proposed for discharge in waters of the U.S. - Testing manual. EPA-823-B-98-004, Washington, D.C.

DEFINITIONS

The following definitions of words and terms are specific to the use of this manual and, where applicable, are quoted verbatim from the Guidelines (cf. Definitions at 40 CFR 230.3 and/or other parts; such definitions are starred*). Thorough familiarization with the following definitions is required prior to use of this manual.

Accuracy: The ability to obtain a true value; determined by the degree of agreement between an observed value and an accepted reference value.

Acid volatile sulfide (AVS): The sulfides removed from sediment by cold acid extraction, consisting mainly of H_2S and FeS. AVS is a possible predictive tool for divalent metal sediment toxicity.

Acute: Having a sudden onset, lasting a short time.

Acute toxicity: Short-term toxicity to organism(s) that have been affected by the properties of a substance, such as contaminated sediment. The acute toxicity of a sediment is generally 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.

***Adjacent:** Bordering, contiguous or neighboring. Wetlands separated from other waters of the United States by man-made dikes or barriers, natural river berms, beach dunes and the like are "adjacent wetlands".

Application factor (AF): A numerical, unitless value, calculated as the threshold chronically toxic concentration of a test substance divided by its acutely toxic concentration. The AF is usually reported as a range and is multiplied by the median lethal concentration as determined in a short-term (acute) toxicity test to estimate an expected no-effect concentration under chronic exposure.

Benchmark organism: Test organism designated by USACE and EPA as appropriately sensitive and useful for determining biological data applicable to the real world. Test protocols with such organisms are published, reproducible and standardized.

Bioaccumulation: The accumulation of contaminants in the tissue of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, pore water or dredged material. [The regulations require that bioaccumulation be considered as part of the environmental evaluation of dredged material proposed for disposal. This consideration involves predicting whether there will be a cause-and-effect relationship between an organism'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].

Bioaccumulation factor: The degree to which an organism accumulates a chemical compared to the source. It is a dimensionless number or factor derived by dividing the concentration in the organism by that in the source.

Bioassay: A bioassay is a test using a biological system. It involves exposing an organism to a test material and determining a response. There are two major types of bioassays differentiated by response: **toxicity tests** which measure an effect (e.g., acute toxicity, sublethal/chronic toxicity) and **bioaccumulation tests** which measure a phenomenon (e.g., the uptake of contaminants into tissues).

Bioavailable: Can affect organisms.

Bioconcentration: Uptake of a substance from water.

Biomagnification: Bioaccumulation up the food chain, e.g., the route of accumulation is solely through food. Organisms at higher trophic levels will have higher body burdens than those at lower trophic levels.

Biota sediment accumulation factor: Relative concentration of a substance in the tissues of an organism compared to the concentration of the same substance in the sediment.

Bulk sediment chemistry: Results of chemical analyses of whole sediments (in terms of wet or dry weight), without normalization (e.g., to organic carbon, grain-size, acid volatile sulfide).

Can: Is used to mean "is able to".

Chronic: Involving a stimulus that is lingering or which continues for a long time.

Chronic toxicity: See **sublethal/chronic toxicity**.

Comparability: The confidence with which one data set can be compared to others and the expression of results consistent with other organizations reporting similar data. Comparability of procedures also implies using methodologies that produce results comparable in terms of precision and bias.

Completeness: A measure of the amount of valid data *obtained* versus the amount of data originally *intended* to be collected.

Confined disposal: A disposal method that isolates the dredged material from the environment. Confined disposal is placement of dredged material within diked confined disposal facilities via pipeline or other means.

Confined disposal facility (CDF): A diked area, either in-water or upland, used to contain dredged material. The terms confined disposal facility (CDF), dredged material containment area, diked disposal facility, and confined disposal area are used interchangeably.

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

***Contaminant:** A chemical or biological substance in a form that can be incorporated into, onto or be ingested by and that harms aquatic organisms, consumers of aquatic organisms, or users of the aquatic environment, and includes but is not limited to the substances on the 307(a)(1) list of toxic pollutants promulgated on January 31, 1978 (43 FR 4109). [Note: A contaminant that causes actual harm is technically referred to as a pollutant, but the regulatory definition of a "pollutant" in the Guidelines is different, reflecting the intent of the CWA.]

Contaminant of concern: A contaminant present in a given sediment thought to have the potential for unacceptable adverse environmental impact due to a proposed discharge.

Control sediment: A sediment essentially free of contaminants and which is used routinely to assess the acceptability of a test. Control sediment may be the sediment from which the test organisms are collected or a laboratory sediment, provided the organisms meet control standards. 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 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.

Data quality indicators: Quantitative statistics and qualitative descriptors which are used to interpret the degree of acceptability or utility of data to the user; include bias (systematic error), precision, accuracy, comparability, completeness, representativeness, detectability and statistical confidence.

Data quality objectives (DQOs): Qualitative and quantitative statements of the overall uncertainty that a decision maker is willing to accept in results or decisions derived from environmental data. DQOs provide the framework for planning environmental data operations consistent with the data user's needs.

Discharge of dredged material: Any addition of dredged material into waters of the United States. [Dredged material discharges include: open water discharges; discharges resulting from unconfined disposal operations (such as beach nourishment or other beneficial uses); discharges from confined disposal facilities which enter waters of the United States (such as effluent, surface runoff, or leachate); and, overflow from dredge hoppers, scows, or other transport vessels]. Material resuspended during normal dredging operations is considered "de minimus" and is not regulated under Section 404 as a dredged material discharge. See 33 CFR 323.2 for a detailed definition. The potential impact of resuspension due to dredging can be addressed under NEPA.

***Disposal site:** That portion of the "waters of the United States" where specific disposal activities are permitted and consist of a bottom surface area and any overlying volume of water. In the case of wetlands on which surface water is not present, the disposal site consists of the wetland surface area. [Note: upland locations, although not mentioned in this definition in the Regulations, can also be disposal sites].

District: A USACE administrative area.

***Dredged material:** Material that is excavated or dredged from waters of the United States. [A general discussion of the nature of dredged material is provided by Engler et al. (1991a)].

EC_{50}: The median effective concentration. The concentration of a substance that causes a specified effect (generally sublethal rather than acutely lethal) in 50% of the organisms tested in a laboratory toxicity test of specified duration.

Elutriate: Material prepared from the sediment dilution water and used for chemical analyses and toxicity testing. Different types of elutriates are prepared for two different procedures as noted in this manual.

Evaluation: The process of judging data in order to reach a decision.

***Factual determination**: A determination in writing of the potential short-term or long-term effects of a proposed discharge of dredged or fill material on the physical, chemical and biological components of the aquatic environment in light of Subparts C-F of the Guidelines.

Federal Standard: The dredged material disposal alternative(s) identified by the U.S. Army Corps of Engineers that represent the least costly, environmentally acceptable alternative(s) consistent with sound engineering practices and which meet the environmental standards established by the 404(b)(1) evaluation process. [See Engler et al. (1988) and 33 CFR 335-338].

***Fill material:** Any material used for the primary purpose of replacing an aquatic area with dry land or changing the bottom elevation of a water body for any purpose. The term does not include any pollutant discharged into the water primarily to dispose of waste, as that activity is regulated under Section 402 of the Clean Water Act. [Note: dredged material can be used as fill material].

Grain-size effects: Mortality or other effects in laboratory toxicity tests due to sediment granulometry, not chemical toxicity. [It is clearly best to use test organisms which are not likely to react to grain-size but, if this is not reasonably possible, then testing must account for any grain-size effects.]

Guidelines: Substantive environmental criteria by which proposed discharges of dredged material are evaluated. CWA Section 404(b)(1) final rule (40 CFR 230) promulgated December 24, 1980.

LC_{50}: The median lethal concentration. The concentration of a substance that kills 50% of the organisms tested in a laboratory toxicity test of specified duration.

Leachate: Water or any other liquid that may contain dissolved (leached) soluble materials, such as organic salts and mineral salts, derived from a solid material.

Lethal: Causing death.

Loading density: The ratio of organism biomass or numbers to the volume of test solution in an exposure chamber.

Management actions: Those actions considered necessary to rapidly render harmless the material proposed for discharge (e.g., non-toxic, non-bioaccumulative) and which may include containment in or out of the waters of the U.S. (see 40 CFR Subpart H). Management actions are employed to reduce adverse impacts of proposed discharges of dredged material.

Management unit: A manageable, dredgeable unit of sediment which can be differentiated by sampling and which can be separately dredged and disposed within a larger dredging area. Management units are not differentiated solely on physical or other measures or tests but are also based on site- and project-specific considerations.

May: Is used to mean "is allowed to".

Method detection limit (MDL): The minimum concentration of a substance which can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

Might: Is used to mean "could possibly."

***Mixing zone:** A limited volume of water serving as a zone of initial dilution in the immediate vicinity of a discharge point where receiving water quality may not meet quality standards or other requirements otherwise applicable to the receiving water. [The mixing zone may be defined by the volume and/or the surface area of the disposal site or specific mixing zone definitions in State water quality standards].

Must: In this manual refers to requirements that have to be addressed in the context of compliance with the Guidelines.

Open water disposal: Placement of dredged material in rivers, lakes or estuaries via pipeline or surface release from hopper dredges or barges.

Pathway: In the case of bioavailable contaminants, the route of exposure (e.g., water, food).

***Pollution:** The man-made or man-induced alteration of the chemical, physical, biological or radiological integrity of an aquatic ecosystem. [See definition of **contaminant**].

***Practicable:** Available and capable of being done after taking into consideration cost, existing technology, and logistics in light of overall project purposes.

Practical quantitation limit (PQL): The lowest concentration that can be reliably quantified with specified limits of precision and accuracy during routine laboratory operating conditions.

Precision: The ability to replicate a value; the degree to which observations or measurements of the same property, usually obtained under similar conditions, conform to themselves. Usually expressed as standard deviation, variance or range.

QA: Quality assurance, the total integrated program for assuring the reliability of data. A system for integrating the quality planning, quality control, quality assessment, and quality improvement efforts to meet user requirements and defined standards of quality with a stated level of confidence.

QC: Quality control, the overall system of technical activities for obtaining prescribed standards of performance in the monitoring and measurement process to meet user requirements.

Reason to believe: Subpart G of the 404(b) (1) guidelines requires the use of available information to make a preliminary determination concerning the need for testing of the material proposed for dredging. This principle is commonly known as "reason to believe", and is contained in Tier I of the tiered testing framework. The decision to not perform additional testing based on prior information must be documented, in order to provide a "reasonable assurance that the proposed discharge material is not a carrier of contaminants" (230.60(b)).

Reference sediment: Point of comparison for evaluating test sediment. Testing requirements in the Section 404(b)(1) Guidelines regarding the point of comparison for evaluating proposed discharges of dredged material are being updated to provide for comparison to a "reference sediment" as opposed to sediment from the disposal site. Because subsequent discharges at a disposal site could adversely impact the point of comparison, adoption of a reference sediment that is unimpacted by previous discharges of dredged material will result in a more scientifically sound evaluation of potential individual and cumulative contaminant-related impacts. This change to the Guidelines was proposed in the Federal Register in January 1995, public comments have been received, and a final rule Notice is being prepared. It is expected that the final rule will be published prior to July 1, 1998, and as a result the reference sediment approach will be implemented in the ITM.

Reference site: The location from which **reference sediment** is obtained.

Region: An EPA administrative area.

region: A geographical area.

Regulations: Procedures and concepts published in the Code of Federal Regulations for evaluating the discharge of dredged material into waters of the United States.

Representativeness: The degree to which sample data depict an existing environmental condition; a measure of the total variability associated with sampling and measuring that includes the two major error components: systematic error (bias) and random error. Sampling representativeness is accomplished through proper selection of sampling locations and sampling techniques, collection of sufficient number of samples, and use of appropriate subsampling and handling techniques.

Sediment: Material, such as sand, silt, or clay, suspended in or settled on the bottom of a water body.

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

Standard operating procedure (SOP): A written document which details an operation, analysis, or action whose mechanisms are thoroughly prescribed and which is commonly accepted as the method for performing certain routine or repetitive tasks.

Standardized: In the case of methodology, a published procedure which has been peer reviewed (e.g., journal, technical report), and generally accepted by the relevant technical community of experts.

Sublethal: Not directly causing death; producing less obvious effects on behavior, biochemical and/or physiological function, histology of organisms.

Sublethal/chronic toxicity: Biological tests which use such factors as abnormal development, growth and reproduction, rather than solely lethality, as end-points. These tests involve all or at least an important, sensitive portion of an organism's life-history. A sublethal endpoint may result either from short-term or long-term (chronic) exposures.

Target detection limit: A performance goal set by consensus between the lowest, technically feasible, detection limit for routine analytical methods and available regulatory criteria or guidelines for evaluating dredged material. The target detection limit is, therefore, equal to or greater than the lowest amount of a chemical that can be reliably detected based on the variability of the blank response of routine analytical methods. However, the reliability of a chemical measurement generally increases as the concentration increases. Analytical costs may also be lower at higher detection limits. For these reasons, a target detection limit is typically set at not less than 10 times lower than available dredged material guidelines.

Tests/testing: Specific procedures which generate biological, chemical, and/or physical data to be used in evaluations. The data are usually quantitative but may be qualitative (e.g., taste, odor, organism behavior). Testing for discharges of dredged material in waters of the United States is specified at 40 CFR 230.60 and 230.61 and is implemented through the procedures in this manual.

Tiered approach: A structured, hierarchical procedure for determining data needs relative to decision-making, which involves a series of tiers or levels of intensity of investigation. Typically, tiered testing involves decreased uncertainty and increased available information with increasing tiers. This approach is intended to ensure the maintenance and protection of environmental quality, as well as the optimal use of resources. Specifically, least effort is required in situations where clear determinations can be made of whether (or not) unacceptable adverse impacts are likely to occur based on available information. Most effort is required where clear determinations cannot be made with available information.

Toxicity: see **Acute toxicity**; **Sublethal/chronic toxicity**, **Toxicity test**.

Toxicity test: A **bioassay** which measures an effect (e.g., acute toxicity, sublethal/chronic toxicity). Not a **bioaccumulation test** (see definition of **bioassay**).

Water quality certification: A state certification, pursuant to Section 401 of the Clean Water Act, that the proposed discharge of dredged material will comply with the applicable provisions of Sections 301, 303, 306 and 307 of the Clean Water Act and relevant State laws. Typically this certification is provided by the affected State. In instances where the State lacks jurisdiction (e.g., Tribal Lands), such certification is provided by EPA or the Tribe (with an approved certification program).

Water quality standard: A law or regulation that consists of the beneficial designated use or uses of a water body, the numeric and narrative water quality criteria that are necessary to protect the use or uses of that particular water body, and an anti-degradation statement.

Waters of the U.S.: In general, all waters landward of the baseline of the territorial sea and the territorial sea. Specifically, all waters defined in Section 230.3 (s) of the Guidelines. [See Appendix A].

Whole sediment: The sediment and interstitial waters of the proposed dredged material or reference sediment that have had minimal manipulation. 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 water to facilitate homogenizing or compositing sediments may be necessary to conducting bioassay tests. These procedures are considered unlikely to substantially alter chemical or toxicological properties of the respective whole sediments except in the case of AVS (acid volatile sulfide) measurements (EPA, 1991a) which are not presently required. 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.

LIST OF ACRONYMS

AAS - Atomic Absorption Spectrometry
AF - Application Factor
AVS - Acid Volatile Sulfide
BAF - Bioaccumulation Factor
BCF - Bioconcentration Factor
BSAF - Biota Sediment Accumulation Factor
CDF - Confined Disposal Facility
CFR - Code of Federal Regulations
CLP - Contract Laboratory Program
CWA - Clean Water Act
ECD - Electron Capture Detection
EO - Executive Orders
EPA - Environmental Protection Agency
FDA - Food and Drug Administration
FR - Federal Register
GC - Gas Chromatography
GFAAS - Graphite Furnace Atomic Absorption Spectrometry
IAEA - International Atomic Energy Agency
ICP - Inductively Coupled Plasma
ITM - Inland Testing Manual
LBP - Lipid Bioaccumulation Potential
MPRSA - Marine Protection, Research and Sanctuaries Act
MS - Mass Spectrometry
NBS - National Bureau of Standards
NEPA - National Environmental Policy Act
NIST - National Institute for Standards and Technology
NOAA - National Oceanic Atmospheric Administration
NPDES - National Pollutant Discharge Elimination System
NRC - National Research Council of Canada
PAH - Polynuclear Aromatic Hydrocarbons
PCB - Polychlorinated Biphenyl
QA - Quality Assurance

QC - Quality Control
QSAR - Quantitative Structure Activity Relationship
RHA - Rivers and Harbors Act of 1899
SAB - Science Advisory Board
SIM - Selected Ion Monitoring
SOP - Standard Operating Procedure
SQC - Sediment Quality Criteria
SQS - Sediment Quality Standards
SRM - Standard Reference Material
TBP - Theoretical Bioaccumulation Potential
TDL - Target Detection Limit
TEF - Toxicity Equivalency Factor
TOC - Total Organic Carbon
TIE - Toxicity Identification Evaluation
USACE - U.S. Army Corps of Engineers
USCS - Unified Soil Classification System
WQC - Water Quality Criteria
WQS - Water Quality Standards

CONVERSIONS

METRIC TO IMPERIAL	IMPERIAL TO METRIC
WEIGHT:	
1Kg = 1000g = 2.205lb	1lb = 16 oz = 0.4536Kg
1g = 1000mg = 2.205 x 10^{-3}lb	
1 mg = 1000µg = 2.205 x 10^{-6}lb	
LENGTH:	
1m = 100cm = 3.28 ft. = 39.370in	1 foot (ft) = 12in = 0.3048m
1cm = 10mm = 0.3937in	
1mm = 1000µg = 0.03937in	
CONCENTRATION:	
1ppm = 1mg/L = 1mg/Kg =1µg/g = 1mL/m^3	1 lb/gal = 7.481lb/ft^3 = 0.120g/cc = 119.826g/L = 119.826Kg/m^3
1g/cc = 1Kg/L = 8.3454 lb/gallon (US)	
1g/m^3 = 1mg/L = 6.243 x 10^{-5}lb/ft^3	1 oz/gal = 7.489Kg/m^3
VOLUME:	
1L = 1000mL	1yd^3 = 27ft^3 = 764.555 L = 0.7646m^3
1mL = 1000µL	1 acre-ft = 1233.482m^3
1cc = $10^{-6}m^3$	1 gallon (US) = 3785cc
	1 ft^3 = 0.0283m^3= 28.3168 L
FLOW:	
1m/s = 196.850 ft/min = 3.281 ft/s	1 ft^3/s = 1699.011 L/min = 28.317 L/s
1 m^3/s = 35.7 ft^3/s	1 ft^2/hr = 2.778 x 10^{-4} ft^2/s = 2.581 x $10^{-5}m^2$/s
	1 ft/s = 0.03048m/s
	1 yd^3/min = 0.45ft^3/s
	yd^3/s = 3.366 gal/s = 12.743 L/s
AREA:	
1 m^2 = 10.764ft^2	1 ft^2 = 0.0929m^2
1 hectare (ha) = 10000m^2 = 2.471 acres	1 acre = 4046.856m^2 = 0.405 ha

PART I - GENERAL CONSIDERATIONS

1.0 INTRODUCTION

1.1 Background

The "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual", commonly referred to as the Inland Testing Manual, updates and replaces "Ecological Evaluation of Proposed Discharge of Dredged or Fill Material into Navigable Waters" (USACE, 1976). This updated manual contains technical guidance for determining the potential for contaminant-related impacts associated with the discharge of dredged material in waters regulated under Section 404 of the CWA (inland waters, near coastal waters, and surrounding environs) through chemical, physical, and biological evaluations. The technical guidance in the manual is intended for use by Army Corps of Engineers (USACE) and Environmental Protection Agency (EPA) personnel, state regulatory personnel, as well as dredging permit applicants and others (e.g., scientists, managers, and other involved or concerned individuals). The results obtained will be utilized within the context of regulatory requirements (discussed in the following sections), to facilitate decision-making with regard to the management of dredged material.

Key changes to the 1976 testing protocol include a tiered testing approach, accommodation for sediment quality standards (SQS), 28-d bioaccumulation testing, comparison of benthic test results with those of the reference sediment, improved statistics, improved model applications, and new test organisms. Because this manual is national in scope, the guidance provided is generic and may need to be modified in certain instances. Application of this guidance in some site- and case-specific situations will require best professional judgment, appropriately documented. Permit applicants and others are strongly encouraged to consult with their appropriate Regional and District experts for additional guidance.

1.2 Statutory/Regulatory Overview

The following sections provide a discussion of the statutory and regulatory framework of the Federal programs within which decisions regarding the management of dredged material discharge activities are made.

1.2.1 Statutory Overview

The USACE and EPA share the Federal responsibility for regulating the discharge of dredged material. The Clean Water Act (CWA) governs discharges of dredged material into "waters of the United States",

including all waters landward of the baseline of the territorial sea. The Marine Protection, Research, and Sanctuaries Act (MPRSA) governs the transportation of dredged material seaward of the baseline (in ocean waters) for the purpose of disposal. In addition, all activities regulated by these statutes must comply with the applicable requirements of the National Environmental Policy Act (NEPA), as well as other Federal laws, regulations and Executive Orders which apply to activities involving the discharge of dredged material.

The CWA was enacted by Congress to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters." The CWA created three permit programs, under Section 401 (as a certification), Section 402 and Section 404, to regulate the point-source discharge of pollutants into waters of the U.S. EPA administers Section 402 which established the National Pollutant Discharge Elimination System (NPDES) Program to regulate discharges of chemicals, heavy metals, and biological wastes, primarily in waste water from industrial processes, publicly owned sewage treatment works, and stormwater discharges. The Section 402 program may be delegated by EPA to the States to administer. EPA and USACE each administer specific aspects of Section 404 which established a permit program and technical guidelines to regulate discharges of dredged or fill material (dredged material and fill material disposal sites must be "specified"). States may assume (and most of them have) the program administered by EPA under Section 401 and must grant, deny, or waive certification for activities permitted or conducted by USACE based on the potential impacts to water quality which may result from a discharge of dredged or fill material to waters of the U.S.

The USACE also administers a regulatory program under Section 10 of the Rivers and Harbors Act of 1899 (RHA) which regulates dredging and other construction activities in navigable waters. The USACE also operates a Federal Civil Works navigation program in conjunction with the CWA and with requirements established within Congressional authorization and appropriation statutes, which involves extensive dredging and dredged material discharge activities. These USACE programs are operated in accordance with NEPA which requires, among other things, the analysis and documentation of potential primary and secondary impacts, including those associated with dredging and dredged material discharges.

1.2.2 Section 404 Regulatory Overview

The USACE has the primary responsibility for the Section 404 regulatory permit program [the USACE regulatory program also administers Section 10 RHA, as well as Section 103 of the MPRSA (for the transport of dredged material to the ocean for the purpose of disposal)] and is authorized, after notice and opportunity for public comment, to issue permits specifying sites for the discharge of dredged or fill material. EPA has the primary role in developing the environmental guidelines, in conjunction with

USACE [the Section 404(b)(1) Guidelines (Guidelines)], by which permit applications must be evaluated. EPA is also responsible for commenting on proposed USACE permits, prohibiting discharges with unacceptable adverse aquatic environmental impacts, approving and overseeing State assumption of the program, establishing jurisdiction, and interpreting exemptions. Both USACE and EPA share enforcement authority.

The USACE regulates the discharge of dredged material, resulting from navigation dredging, into waters of the United States. The USACE also regulates the discharge of dredged material and incidental discharges of dredged material resulting from mechanized landclearing, ditching, channelization, and other excavation activities. The Inland Testing Manual has been developed to facilitate testing in conjunction with proposed dredged material discharges resulting from navigation dredging. The testing protocols are not designed or intended to be applied to discharge of dredged material and incidental discharges of dredged material resulting from mechanized landclearing, ditching, channelization, and other excavation activities, except where excavation and subsequent discharge activities are of essentially the same character as those associated with navigation dredging and disposal (e.g., open water discharges of dredged material excavated from a soft-bottom flood control channel or reservoir).

The USACE's evaluation of a Section 404 permit application involves determining whether the proposed project complies with the Guidelines (40 CFR 230) and USACE permit regulations (33 CFR 320-330) which require a public interest review of the project. [Public interest factors (listed in 33 CFR 320.4) considered with respect to dredged material contaminant-related impacts include water quality, water supply and conservation, safety, and fish and wildlife impacts]. A permit is issued provided the proposed project complies with the Guidelines and is not contrary to the public interest. The USACE issues individual permits and general permits. Individual permits are issued on a project-by-project basis after the Guidelines compliance and public interest determinations are made for the specific project at issue. General permits, on the other hand, are issued for classes of activities after the USACE conducts the Guidelines compliance and public interest reviews and determines that issuance of the general permit will not result in more than minimal adverse impacts to the aquatic environment from either a site-specific or cumulative standpoint. General permits require little or no reporting, analysis, or paperwork.

There are three types of general permits issued by the USACE, nationwide permits, regional general permits and programmatic general permits. Nationwide permits are issued by the Chief of Engineers and apply nationwide. Regional permits are issued by District and Division Engineers and are applicable on district or State-wide basis. Programmatic permits are issued (by the Chief of Engineers, as well as District and Division Engineers) to other federal, State or local agencies with the intention of providing the appropriate level of environmental protection and avoiding unnecessary duplication of effort with the agency regulatory activities at issue.

There are currently four nationwide permits that pertain to dredging and the discharge of dredged material. One authorizes the discharge and return water from confined disposal areas (provided the associated dredging is authorized pursuant to Section 10 of the River and Harbor Act of 1899); two other nationwide permits authorize the dredging and discharge, respectively, of up to 25 cubic yards of material; and a fourth authorizes maintenance dredging of existing marina basins (provided that the dredged material is deposited on uplands; return water from a confined disposal area requires separate authorization pursuant to Section 404 of the Clean Water Act). The USACE depends on its districts' knowledge of potentially contaminated areas and on the discretionary authority of District and Division Engineers to develop special conditions and/or require individual permits where contaminated sediments are present. General permits are not intended to apply to projects involving the dredging or the discharge of contaminated materials.

USACE Civil Works activities are conducted in accordance with the Guidelines and the USACE operation and maintenance regulations (33 CFR 335-338). The USACE specifies sites for the discharge of dredged material in conjunction with its regulatory and civil works responsibilities. (Permits are not actually issued in conjunction with USACE discharge activities).

1.2.2.1 The Section 404(b)(1) Guidelines

The Guidelines provide the substantive environmental criteria used in evaluating proposed discharges of dredged or fill material into waters of the United States. Fundamental to these Guidelines is the precept that dredged or fill material should not be discharged into the aquatic ecosystem, unless it can be demonstrated that such a discharge will not have an unacceptable adverse impact either individually or in combination with known and/or probable impacts of other activities affecting the ecosystems of concern.

For proposed discharges of dredged material to comply with the Guidelines, they must satisfy four requirements found in Section 230.10 as follows. Section 230.10(a) addresses those impacts associated with the loss of aquatic site functions and values of the proposed discharge site, by requiring that the discharge site represent the least environmentally damaging, practicable alternative. Section 230.10(b) requires compliance with established legal standards (e.g., issuance or waiver of a State water quality certification). Section 230.10(c) requires that discharge of dredged material not result in significant degradation of the aquatic ecosystem. Section 230.10(d) requires that all practicable means be utilized to minimize for adverse environmental impacts.

Testing as described in this manual is part of the larger evaluation of a proposed discharge activity to

determine its compliance with the Guidelines. Sections 230.60 and 230.61 of the Guidelines provide the basis for certain factual determinations with regard to dredged material discharge activities. Section 230.60 provides for a general evaluation of the material and establishes a framework to determine, based on existing information on the proposed dredging and discharge sites, whether the material at issue requires further testing. If the conditions at 230.60 cannot be met or are not applicable, the testing requirements of Section 230.61 must be applied. This manual details the testing procedures outlined in 230.60 and 230.61. Conclusions reached utilizing this manual will be used to make factual determinations of the potential effects of a proposed discharge of dredged or fill material on the physical, chemical and biological components of the aquatic environment. Such factual determinations are used to make findings of compliance or noncompliance with relevant parts of Sections 230.10(b) (including compliance with established water quality standards) and 230.10(c) (determinations of potential contaminant-related impacts to aquatic resources). All specifications of discharge sites must also comply with Section 230.10 (a) and Section 230.10(d). Site monitoring and/or management activities developed following the use of this manual may be said to contribute to satisfying the aforementioned requirements of Section 230.10(d).

Once compliance with the Guidelines is established, information developed utilizing the manual will also be factored into the USACE public interest determination which is required by its regulatory permit regulations for proposed non-Federal dredged material discharge activities, or its determinations required by the operation and maintenance regulations pertaining to Federal Civil Works activities. In making determinations with regard to its regulatory and civil works responsibilities, the USACE considers a continuum of discharge options, on a project-specific basis, including alternative sites, mitigation and specific site management and monitoring conditions. Determination of whether a material, which would not otherwise comply with the Guidelines or with other USACE regulatory and civil works requirements, could be brought into compliance through appropriate management actions or other discharge methods, is beyond the scope of this manual.

1.2.2.2 Particulars of Sections 230.60 and 230.61

Reason to Believe - Subpart G of the 404(b)(1) guidelines requires the use of available information to make a preliminary determination concerning the need for testing of the material proposed for dredging. This principle is commonly known as "reason to believe". The decision to not perform testing based on prior information must be documented in order to provide a "reasonable assurance that the proposed discharge material is not a carrier of contaminants" (by virtue of the fact that it is sufficiently removed from sources of pollution) [230.60(b)]. The reason to believe that no testing is required is based on the type of material to be dredged and/or its potential to be contaminated. For example, dredged material is most likely to be free of contaminants if the material is composed primarily of sand, gravel, or other

inert material and is found in areas of high current or wave energy [230.60(a)]. In addition, knowledge of the proposed dredging site proximity to other sources of contamination, as well as that gained from previous testing or through experience and knowledge of the area to be dredged, may be utilized to conclude that there is no reason to believe that contaminants are present [230.60(b)] and, therefore, no need for testing. This general evaluation comprises procedures found in Tier I of the manual's tiered-testing framework. Tier I is a comprehensive analysis of all existing and readily available information on the proposed dredging project, including all previously collected physical, chemical, and biological data for both the proposed dredging and discharge sites. A more complete discussion of technical factors to consider with respect to Sections 230.60(a) and (b) in Tier I is provided in Section 4.0.

Exclusions From Testing - Sections 230.60(c) and (d) provide for specific circumstances in which the discharge of dredged material which is suspected to be contaminated may be conducted without further testing. Section 230.60(c) provides that where the proposed discharge and dredging sites are adjacent and are comprised of similar materials and subject to the same source(s) of contaminants, disposal may be conducted without further testing because the discharge is not likely to result in degradation of the discharge site, as long as the potential spread of contaminants to less contaminated areas can be prevented. Section 230.60(d) provides that the discharge of contaminated dredged material may be conducted without further testing if constraints, acceptable to USACE and EPA, are available to reduce contamination to acceptable levels within the discharge site, and to prevent contaminants from being transported beyond the proposed discharge site boundaries.

Conclusions reached with regard to dredged material discharges without testing, in accordance with Section 230.60, must be described in the appropriate factual determination. Even though material may be excluded from testing under the manual the water quality certifying agency may require testing to demonstrate compliance with state laws. Even in cases where the discharge site is adjacent to the dredging site, potential differences in contaminant bioavailability may occur.

Reference Sediment - The manual requires comparison of testing results between the proposed dredged material and a reference sediment (see previous Definitions section). The USACE and EPA believe that the use of a reference sediment provides an accurate information base for predicting cumulative bioaccumulation and benthic impacts resulting from the discharge of dredged material.

1.2.3 Relationship to Section 401 CWA Water Quality Certification

Section 401 of the CWA requires that all Federal permits and licenses, including those for the discharge of dredged material into waters of the United States, authorized pursuant to Section 404 of the CWA,

must be certified as complying with applicable State water quality standards (WQS). The Guidelines at 40 CFR 230.10(b) state in part that "No discharge of dredged or fill material shall be permitted if it: (1) Causes or contributes, after consideration of disposal site dilution and dispersion, to violations of any applicable State water quality standard." This applies at the edge of a State designated mixing zone.

The process for adoption of State WQS is prescribed at 40 CFR 131. States must issue, condition, deny, or waive a Water Quality Certification for activities permitted or conducted by USACE, certifying that no adverse water quality impacts will occur based on determinations of compliance with applicable State WQS which have been adopted in accordance with the above regulation. State water quality standards consist of designated uses, narrative and numeric criteria designed to support those uses, and anti-degradation provisions. This testing manual is intended to provide guidance for the dredged material testing necessary to determine compliance with such State WQS.

States may, at their discretion, include in their State standards policies generally affecting their application and implementation, e.g. mixing zones (40 CFR 131.13). A mixing zone is a limited volume of water serving as a zone of initial dilution in the immediate vicinity of a discharge point where receiving water may not meet quality standards or other requirements otherwise applicable to the receiving water (40 CFR 230.3). Where mixing zone provisions are part of the State standards, the State should describe the procedures for defining mixing zones.

According to EPA (1991b), mixing zone concentrations should not exceed acute water quality standards and, considering likely pathways of exposure, there should be no significant human health risks. For dredged material discharges which only occur periodically, water quality standard compliance in the mixing zone is generally focused on aquatic life, not on human health, which is based on long-term exposures to contaminants. (Long-term exposures resulting from accumulations of dredged material at the disposal site can be evaluated by such means as bioaccumulation tests). Acute or chronic standards may be appropriate, depending on the duration of discharge and characteristics of the discharge site.

Many States have statutory or regulatory requirements for use of State-owned lands, including aquatic (marine and freshwater) bedlands. For discharges of dredged or fill materials into waters of the U.S. which are also waters of State or State-owned lands, specific requirements (including testing) for "use" of State lands may exist which need to be considered. The responsible State land-management agency may be different from the agency which normally issues the WQS or coastal zone certification. At a minimum, coordination with the responsible State agency should occur to avoid conflicts with or impacts to existing and/or future uses of State lands. In parts of the country, cooperative State-federal dredged material or sediment management ventures are in place or are being pursued to identify disposal sites, develop consistent regional management standards, and to monitor maintenance of those standards [e.g.,

the Puget Sound Dredged Material Disposal Analysis (State of Washington) and San Francisco Long-Term Management Strategy (LTMS - State of California)]. These programs are intended to streamline the regulatory process associated with dredging and dredged material disposal.

2.0 SCOPE AND APPLICABILITY

This manual is directed towards evaluation of proposed discharges of dredged material (associated with navigational dredging or dredging activities of essentially the same character as navigational dredging) in open water. It utilizes both chemical and biological analyses as necessary, to provide effects-based conclusions within a tiered framework with regard to the potential for contaminant-related water column, benthic toxicity and benthic bioaccumulation impacts. The tiered-testing procedure detailed in Section 3.1 is comprised of four levels (tiers) of increasing investigative intensity which generate information to assist in making contaminant-related determinations. Tiers I and II use existing or easily acquired information and apply relatively inexpensive and rapid tests to predict environmental effects. Tiers III and IV contain biological evaluations which are more intensive and require field sampling, laboratory testing, and rigorous data analysis.

2.1 This Manual is Intended to Address:

- contaminant-related impacts associated with discharges of dredged material (resulting from navigational dredging or dredging activities of essentially the same character as navigation dredging, such as open water discharges of dredged material excavated from a soft-bottom flood control channel or reservoir) in open water disposal areas, including wetlands.

- contaminant-related impacts to waters of the U.S. associated with dredged material runoff from confined disposal areas. Guidance on evaluation of such discharges is provided in Appendix B.

2.2 This Manual is Not Intended to Address:

- impacts associated with the dredging activity itself.

- impacts associated with dredged material discharges associated with excavation of drainage ditches and landclearing.

- impacts associated with the discharge of fill material. However, where dredged material associated with navigational dredging will be discharged in open water as fill, the procedures of this manual are applicable.

- microbiological impacts except for impacts in conjunction with the State designated use of a waterbody and human health considerations. The manual provides a list of applicable references, as the technology for analyzing other potential impacts from microorganisms (e.g., modeling potential pathways of contamination) is in various stages of development. Although scientifically accepted mechanisms for predicting the degree of potential microbiological impacts are not yet available, site management techniques are available (but are beyond the scope of this manual) to address potential impacts (e.g., aerating dredged material to kill anaerobic organisms).

2.3 Dredged Material Discharge for Beneficial Uses

The testing procedures in this manual should also be applied when navigational dredged material is proposed for certain beneficial uses. To the extent that dredged material will be discharged into open water in conjunction with a beneficial use and the evaluation of its suitability requires analysis of contaminant-related impacts listed in 2.1, the testing protocols of this manual should be applied. However, other evaluations may be necessary, in addition to those in this manual, to assess the potential for contaminant-related impacts through pathways other than those provided by open water. For example, contaminants in dredged material proposed for wetlands creation which will not adversely affect the open water environment, may be taken up by wetlands vegetation, thereby requiring evaluations that are not detailed in this manual.

This manual may also apply to dredged material used for beach nourishment. Beach nourishment normally involves hydraulic or mechanical placement of uncontaminated materials near a shoreline. As with other beneficial uses, dredged material proposed for beach nourishment often can be excluded from chemical or biological testing; the focus is on analysis to determine physical compatibility as measured by grain size and total organic carbon (see Section 9.1). However, if there is a reason to believe that contaminants are present, further evaluation should be performed.

2.4 The Role of Biological Evaluations (Toxicity and/or Bioaccumulation Tests) in the Manual

As noted in Section 230.61 of the Guidelines, the evaluation process will usually entail investigation of potential biological effects, rather than merely chemical presence, of the possible contaminants. Biological evaluations serve to integrate the chemical and biological interactions of the suite of contaminants which may be present in a dredged material sample, including their availability for biological uptake, by

measuring their effects on test organisms. Within the constraints of experimental conditions and the end-points of effects measured, biological evaluations provide for a quantitative comparison of the potential effects of a dredged material when compared to reference sediments. Thus, a specified level of change compared to reference conditions and a statistically significant result in this comparison indicate that the discharge of the dredged material in question may cause a direct and specific biological effect under test conditions and, therefore, has the potential to cause an ecologically undesirable impact. Guidance for the conduct of biological tests is given in Sections 11 and 12.

Dredged material potentially contains a myriad of chemical contaminants which may adversely impact aquatic organisms. The literature is replete with examples where aquatic organism sensitivity varies with the type of contaminant (e.g., see Rand and Petrocelli, 1985) and, as a result, a suite of aquatic species are routinely recommended to fully assess the impact of contaminants on a biological community. In this manual, three sensitive species are recommended for the water column and whole sediment toxicity tests. In the case of the latter, two species can be used, provided they cover three functional characteristics: filter feeder, deposit feeder, burrower. In both cases, at least one of these species must be a sensitive "benchmark" species. For assessing bioaccumulation, adequate tissue biomass and the ability to ingest sediments is more important than taxon sensitivity. Where possible, two species should be used to assess potential bioaccumulation unless adequate regional data are available to justify single species testing.

It is important to recognize that dredged material bioassays (toxicity and bioaccumulation tests) are subject to interpretation and are not precise predictors of environmental effects. This manual does not provide quantitative guidance on interpreting the ecological meaning of such effects (e.g., the ecological consequences of a given tissue concentration of a bioaccumulated contaminant or the consequences of that body burden to the animal). Rather, the manual considers statistically significant increases above certain levels compared to the reference sediment as potentially undesirable. Because a statistically significant difference is not a quantitative prediction that an ecologically important impact would occur in the field or vice versa, this manual discusses additional factors to be weighed in evaluating potential ecological impact. This is more likely to result in environmentally sound evaluations than is reliance on statistical significance alone.

Bioaccumulation evaluations indicate biological availability of contaminants in dredged material, which may bioaccumulate and bioconcentrate in (or, for a few chemicals, biomagnify up) aquatic food webs to levels which might be harmful to consumers, including human beings, without killing the intermediate organisms. To use bioaccumulation data, it is necessary to predict whether there will be a cause-and-effect relationship between the animal's exposure to dredged material and a meaningful adverse elevation of body burden of contaminants above that of similar animals not exposed to the dredged material.

2.5 The Role of Water and Sediment Chemical Evaluations in the Manual

Chemical evaluations of water and sediments are conducted for the following reasons:

- to determine contaminant concentrations in the dredged material
- to determine contaminant concentrations in the discharge or reference sites
- to determine compliance with water quality standards (WQS).

Chemical evaluations may be made on the basis of previous chemical inventories, when there is a reason to believe that the dredged material contains no new contaminants, or that there is no difference between contaminants in the dredged material and the disposal site [Tier I; Section 230.60(a)-(c) of the Guidelines]. The latter may be the case where the discharge site is adjacent to the dredging site, and potential differences in contaminant bioavailability are considered unlikely. There may, however, be concern with potential water column effects which would warrant evaluation of such potential effects (Tier II; Section 2.6). In particular, it must be shown that unacceptable levels of dissolved and suspended contaminants from the discharge either will not be released and transported to less contaminated areas, or can be managed.

Initial evaluation of water column chemistry may be carried out through the use of a numerical dispersion model based on bulk sediment chemistry (Section 5.1.1). If this model indicates the potential for adverse effects, a chemical evaluation of potential water column effects may be conducted through the use of elutriate tests [Tier II; Section 230.61(b)(2) of the Guidelines]. In this procedure an aqueous extract (i.e., an elutriate) is prepared from the material to be discharged, and the dissolved contaminants are compared to water quality standards with consideration of mixing. This comparison requires that dissolved contaminants in reference water (ambient condition) also be analyzed.

The above elutriate test is used to determine compliance with WQS with consideration of mixing. The elutriate test provides an indirect evaluation of potential biological effects, because WQS are derived from toxicity tests of solutions of various contaminants. Even if WQS are met, biological evaluations (see Section 2.4) must be considered.

2.6 Water Column Effects

The dredged material impact in the water column must be within the available WQS for all contaminants of concern outside of the mixing zone. If disposal operations result in long-term exposures, compliance with chronic aquatic and/or human health standards should be evaluated. Wildlife standards, if available, should also be considered. Water column toxicity tests are used to provide information on the toxicity

of contaminants not included in water quality standards, and also to indicate possible interactive effects of multiple contaminants.

2.7 Mixing

Appendix C describes the method to be used for estimating the effect of mixing for water column evaluations. 40 CFR 230.11(f)(2) describes the factors to be considered in defining mixing zones; States may use additional factors in such definition. This method is applied in evaluating the potential for impacts of the portion of dredged material that remains in the water column; all water quality and water column toxicity data must be interpreted in light of mixing [Section 230.61 (b)(2)(ii) of the Guidelines]. This is necessary because biological effects (which are the basis for WQS) are a function of the biologically available contaminant concentration and exposure time of the organisms. Laboratory toxicity tests expose organisms to specific concentrations for fixed periods of time, whereas in the field both concentration and exposure time to contaminants change continuously due to mixing and dilution. Both factors interact to control the degree of biological impact. Thus, it is necessary to incorporate the mixing expected at the discharge site into the interpretation of data.

2.8 Benthic Effects

Generally, the greatest potential for environmental effects from dredged material discharge lies in the benthic environment. 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 living and feeding on deposited material for extended periods represent the most likely pathways by which adverse effects to aquatic biota can occur. Therefore, the major evaluative effort must be placed on deposited material and the benthic environment, unless there is a compelling reason to do otherwise. The approach in this manual is conservative (i.e., protective) as it uses whole-sediment bioassays (toxicity and bioaccumulation tests) to evaluate the solid phase of the dredged material. Sediment chemical analyses currently cannot be used to directly evaluate the biological effects of any contaminants which may be present in dredged material because such potential effects are a function of bioavailability. However, as noted in Section 2.5, there are circumstances where it may be reasonably assumed that bioavailability in the dredged material and the discharge site are similar. When decisions cannot be made using evaluations in Section 230.60 of the Guidelines, bioaccumulation tests should be used to directly determine the bioavailability of potential contaminants.

2.9 Management Options

Some dredged material evaluated in accordance with technical procedures in this manual may demonstrate

a potential for unacceptable environmental impacts or not meet Federally approved State WQS. If so, a careful case-by-case evaluation of management options (e.g., alternative dredging and discharge methods, alternative discharge sites, confined disposal, capping, site controls such as covers and/or liners) will be necessary to determine whether the proposed discharge can be made acceptable or can be brought into compliance with the Guidelines and State WQS. As previously noted, it is beyond the scope of this manual to determine whether a material which would not otherwise comply with the Guidelines, could be brought into compliance through appropriate management actions or other discharge methods.

2.10 The Relationship of the Inland Testing Manual to Other USACE/EPA Dredged Material Management Efforts

2.10.1 Relationship of the Manual to the USACE/EPA Framework Document

EPA and USACE have long recognized the need for a consistent technical framework for decision-making regarding the discharge of dredged material in ocean, near coastal, and inland waters (e.g., see Francingues et al., 1985; Wright and Saunders, 1990). This manual is one of a series of guidance documents jointly developed by EPA and the USACE in response to that recognition. This series of guidance documents includes the "Evaluating Environmental Effects of Dredged Material Management Alternatives - A Technical Framework" (USACE/EPA, 1992) which articulates those factors (including the potential for and degree of contaminant-related impacts) to be considered in identifying the environmental effects of dredged material management alternatives on a continuum from uplands to oceans, and which meet the substantive and procedural requirements of NEPA, CWA and MPRSA. The companion testing manual for ocean disposal, the Green Book (EPA/USACE, 1991) is included in the series. Application of the testing guidance in this manual within the context of the Framework Document will allow for consistency in decision-making with respect to technical considerations, across statutory boundaries and with consideration of the continuum of dredged material discharge options.

2.10.2 Relationship of the Manual to the EPA/USACE Green Book

Although the Ocean Dumping and the CWA programs carry out their functions under different mandates and different environments (estuarine, lake and riverine *versus* ocean), there is a considerable overlap in terms of practical application. The Guidelines are statutorily directed to be based upon criteria comparable to those developed under Section 403(c) for the territorial seas, contiguous zone, and ocean. Additionally, in previous guidance both EPA and USACE have acknowledged the ecological similarity of all aquatic areas and the need for a consistent technological analysis framework, particularly when the waters of the

United States under consideration for a discharge are near-coastal. While details of this manual are necessarily different from one addressing only ocean waters, the tiered testing framework and concepts of the Green Book are an appropriate paradigm. The Inland Testing Manual also utilizes the Green Book's reference site approach which provides a more accurate data base for cumulative impact analysis.

Dredged material transported for purposes of dumping or disposal seaward of the baseline of the territorial sea will continue to be regulated under the MPRSA (commonly referred to as the Ocean Dumping Act). MPRSA-regulated dredged material disposal will be tested in accordance with procedures outlined in the Green Book (EPA/USACE, 1991). As previously discussed, dredged material used as fill within the territorial sea, such as for beach nourishment, is regulated under the CWA and will be tested in accordance with this manual.

2.10.3 Relationship of the Manual to EPA's Contaminated Sediment Strategy and Sediment Quality Criteria

EPA is developing a Contaminated Sediment Management Strategy (Strategy; Southerland et al., 1992) which is a multi-program effort to address contaminated aquatic sediments in the United States. The Strategy is intended to improve the understanding of the extent and severity of sediment contamination and to propose prevention, control, and remediation programs. The Strategy describes the policy framework and specific actions EPA could take to promote the consideration of and reduction of ecological and human health risks posed by sediment contamination. The Strategy also recommends a comprehensive research program and outreach activities with other agencies and the general public.

One component of the Strategy is the development of Sediment Quality Criteria (SQC), which are derived numerical values representing the concentration of chemicals in sediment which are determined to adversely affect benthic organisms. SQC are included in EPA's approach to defining contamination in sediments, and are envisioned to play a range of roles in all programs, from assessment to remediation. When finalized, SQC likely will be incorporated into the Inland Testing Manual in Tier II. SQC could also form the basis for State SQS. The Inland Testing Manual is structured such that evolving science may be readily merged into the document.

PART II - EVALUATION OF POTENTIAL ENVIRONMENTAL IMPACT

3.0 OVERVIEW OF TESTING AND EVALUATION

As noted in Section 1.2.2.1, conclusions reached utilizing this manual will be used to make factual determinations of the potential effects of a proposed discharge of dredged material on the physical, chemical and biological components of the aquatic environment. Such factual determinations are used to make findings of compliance or noncompliance with relevant parts of Sections 230.10(b) (including compliance with established water quality standards) and 230.10(c) (determinations of potential contaminant-related impacts to aquatic resources).

3.1 Tiered Testing and Evaluation

The tiered approach to testing used in this manual must be initiated at Tier I. It is designed to aid in generating physical, chemical, toxicity and bioaccumulation information, but not more information than is necessary to make factual determinations. This allows optimal use of resources by focusing the least effort on disposal 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 (and cost) of investigation. Tiered testing results in environmental protection in the context of more efficient completion of necessary evaluations and reduced costs, especially to low-risk operations. Disposal operations that obviously have low environmental impact generally should not require intensive investigation to make factual determinations. Evaluation at successive tiers is based on more extensive and specific information about the potential impact of the dredged material, that may be more time-consuming and expensive to generate, but that allows more and more comprehensive evaluations of the potential for environmental effects. At any tier except for Tier IV, failure to satisfactorily determine the potential for unacceptable aquatic environmental impact, or to develop sufficient information to make factual determinations, results in additional testing at a subsequent, more complex tier unless a decision is made to seek other disposal alternatives (thereby avoiding the potential for unacceptable aquatic environmental impacts).

It is necessary to proceed through the tiers only until information sufficient to make factual determinations has been obtained. For example, if the available information is sufficient to make factual determinations, no further testing is required.

The initial tier (Tier I) uses readily available, existing information (including all previous testing). For certain dredged materials with readily apparent potential for environmental impact (or lack thereof), information collected in Tier I may be sufficient for making factual determinations. However, more

extensive evaluation (Tiers II, III and IV) may be needed for other materials with less clear potential for impact or for which Tier I information is inadequate.

Tier II is concerned solely with sediment and water chemistry. Tier III is concerned with well-defined, nationally accepted toxicity and bioaccumulation testing procedures. Tier IV allows for case-specific laboratory and field testing, and is intended for use in unusual circumstances.

The approach is to enter Tier I and proceed as far as necessary to make factual determinations. Although it is not always necessary that all dredged material be evaluated through all tiers, there must be enough information available to make determinations on all aspects of the Guidelines relating to water column impact, benthic toxicity and benthic bioaccumulation. It is acceptable to carry water-column and benthic evaluations, or toxicity and bioaccumulation evaluations, to different tiers to generate the information necessary and sufficient to make these determinations.

Prior to initiating testing, it is essential that the informational requirements of preceding tiers be thoroughly understood and that the information necessary for interpreting results 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 even though it may be clear without formal Tier I evaluation that further assessment will be necessary.

The tests in this manual reflect the present state-of-the-art procedures for dredged material evaluation. However, 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 will be integrated into the tiered framework. 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 this manual, in particular Sections 4, 5, 6 and 7. The sections or figures in the manual that present the technical guidance shown by the flowcharts are indicated in the boxes on the figures.

3.2 Control and Reference Sediments

It is important to clearly distinguish between control and reference sediments in the context of testing for benthic impacts. In general, control sediment is that within which the organisms resided prior to

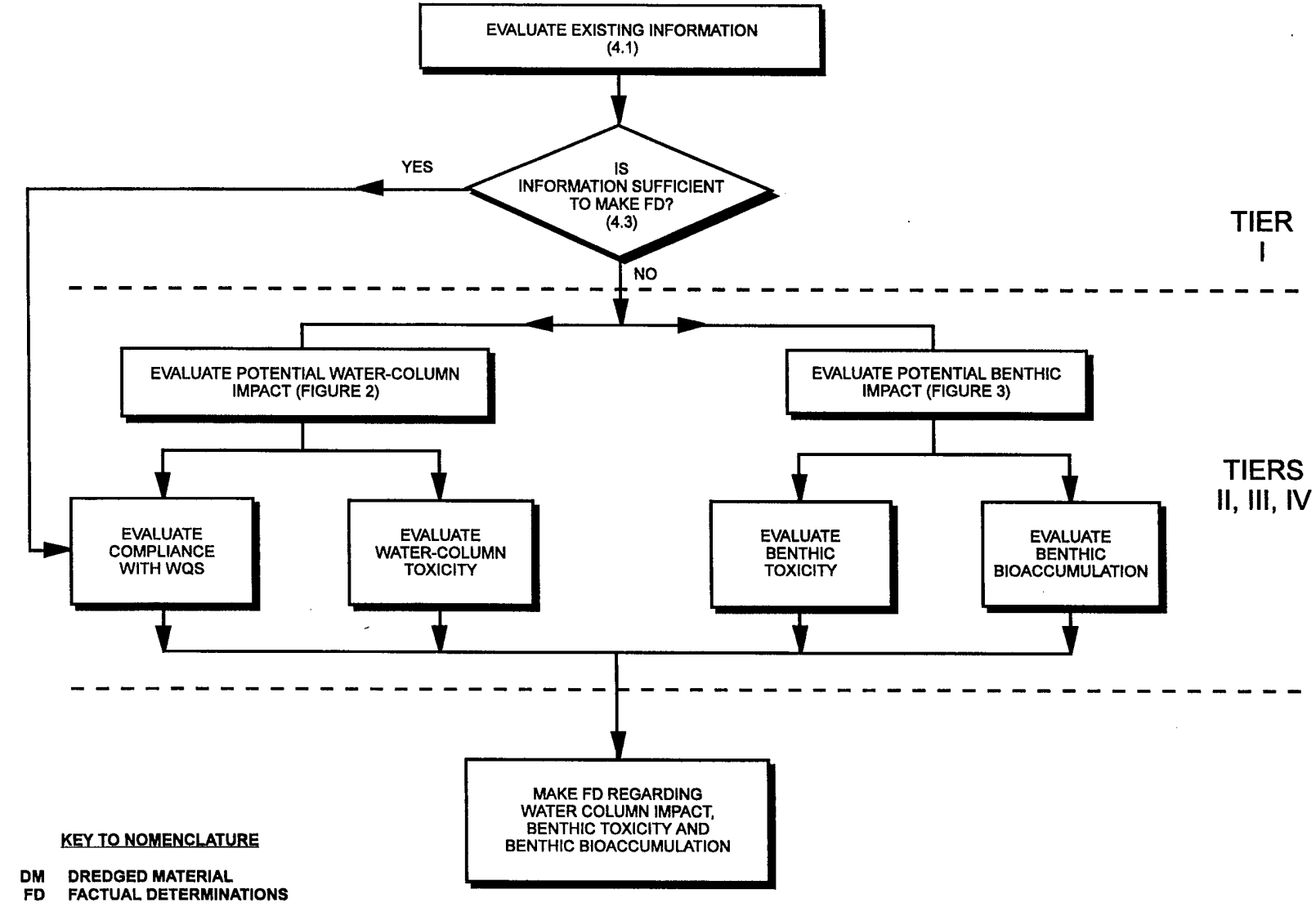


Figure 3-1. Simplified Overview of Tiered Approach to Evaluating Potential Impact of Aquatic Disposal of Dredged Material.

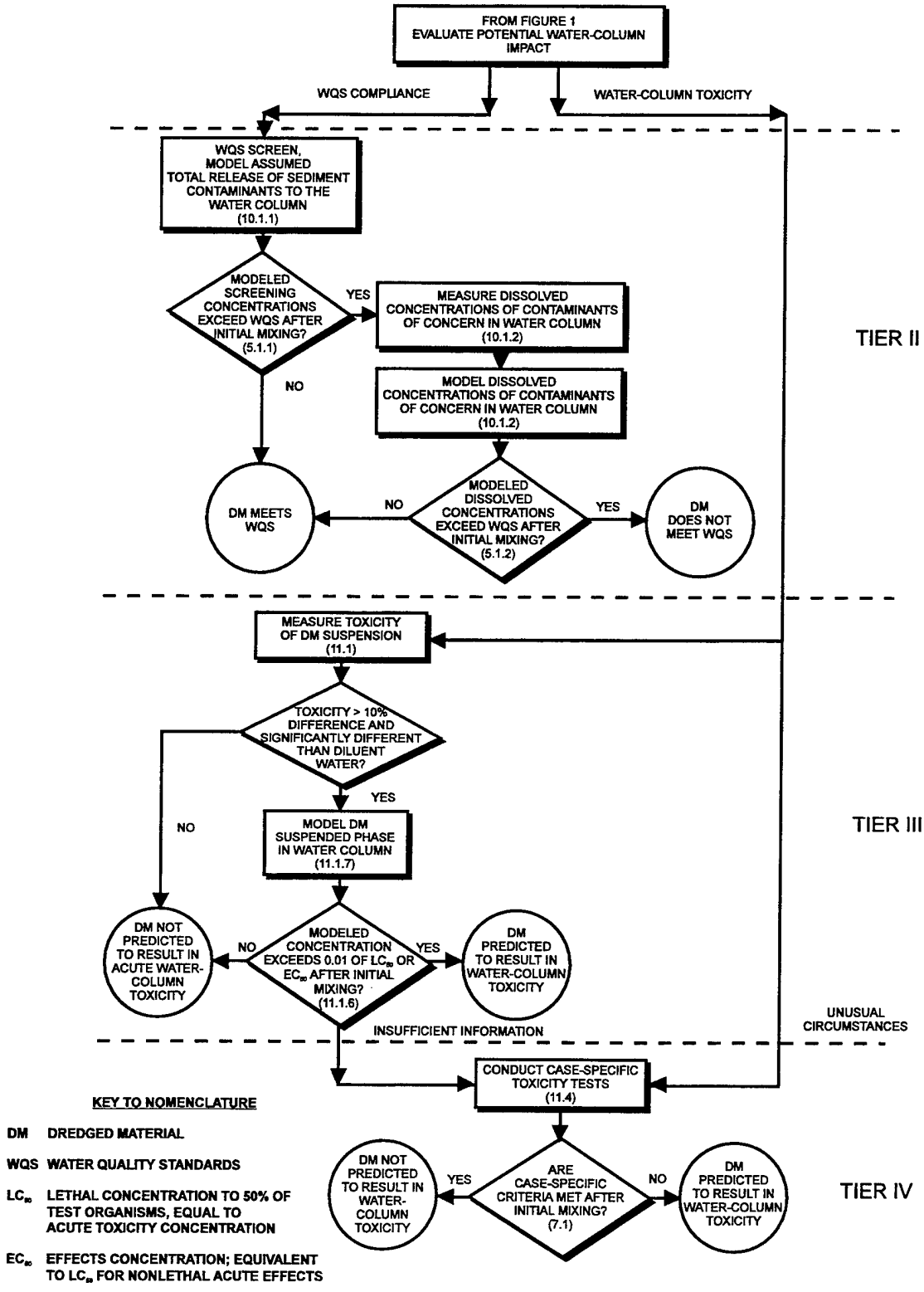


Figure 3-2. Illustration of Tiered Approach to Evaluating Potential Water Column Impacts of Dredged Material.

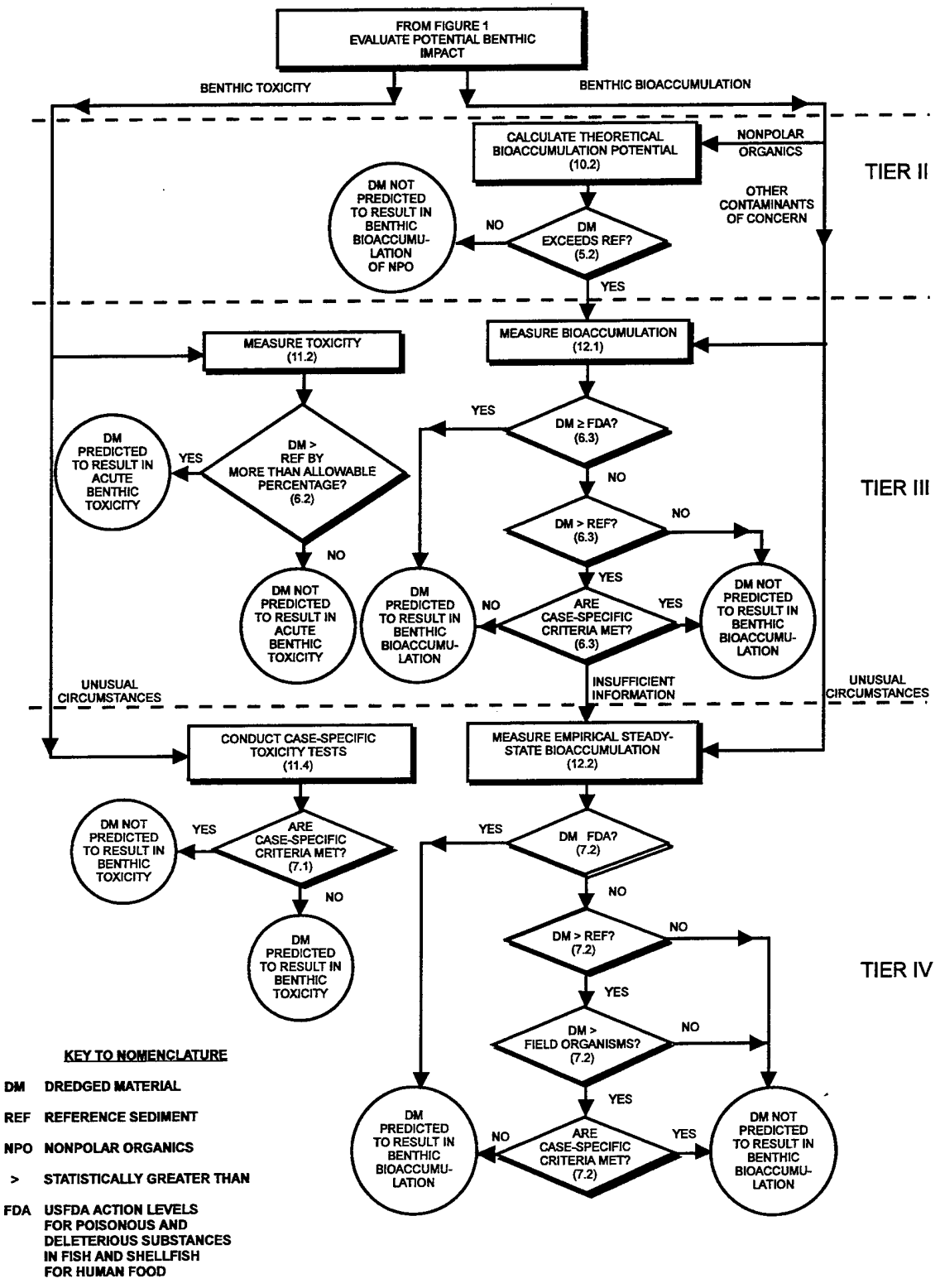


Figure 3-3. Illustration of Tiered Approach to Evaluating Potential Benthic Impacts of Deposited Dredged Material.

collection in the field or is that within which they were cultured in the laboratory, and serves to confirm the health of the test animals and the acceptability of the test conditions. Generic control sediments are also possible and consist of field-collected or laboratory prepared sediment. Reference sediment is the key to the evaluation of dredged material. Results of tests using reference sediment provide the point of comparison (reference point) to which benthic effects of dredged material are compared.

In some cases, it may be appropriate to use more than one reference sediment for a single dredging project. This could occur when the dredged material or the disposal site has a wide range of grain-sizes or TOC, when management needs suggest that disposal of different dredged materials at different locations in the disposal site is desirable, or when discharge at more than one site is being considered. One reference site can serve more than one disposal site.

3.2.1 Reference Sediment Sampling

Reference sediment is the point of comparison for evaluating test sediment. Testing requirements in the Section 404(b)(1) Guidelines regarding the point of comparison for evaluating proposed discharges of dredged material are being updated to provide for comparison to a "reference sediment" as opposed to sediment from the disposal site. Because subsequent discharges at a disposal site could adversely impact the point of comparison, adoption of a reference sediment that is unimpacted by previous discharges of dredged material will result in a more scientifically sound evaluation of potential individual and cumulative contaminant-related impacts. This change to the Guidelines was proposed in the Federal Register in January 1995, public comments have been received, and a final rule Notice is being prepared. It is expected that the final rule will be published prior to July 1, 1998, and as a result the reference sediment approach will be implemented in the ITM.

Reference sediment is generally collected outside the influence of previous disposal operations at a dredged material disposal site, but near enough to the disposal site that the reference sediment is subject to all the same influences (except previously disposed dredged material) as the disposal site. If there is a potential for sediment migration or there is a reason to believe that previously disposed sediment has migrated, reference sediment should be collected from an area that is not expected to be influenced by test material. There are four potential reference sampling approaches as discussed below. We recommend the first two reference approaches because they allow statistically valid comparisons.

Reference Point Approach: This approach is used when the disposal site is known to be sufficiently homogeneous that a single reference location is representative of the disposal site. A single reference location is sampled and the sediment is tested concurrently with the dredged material. The bioassay results from the reference sediment are statistically compared to those obtained from benthic toxicity and bioaccumulation tests of the material to be dredged.

Reference Area Approach: This approach is used when the disposal site is known to be heterogeneous and more than one reference location must be sampled to adequately characterize the disposal site. Several reference locations are sampled and a composite of all of the sediments are tested concurrently with the dredged material. The bioassay results from the reference sediment composite are statistically compared to those obtained from benthic toxicity and bioaccumulation tests of the material to be dredged.

Periodic Reference Point Approach: This approach could, theoretically, be used when it is not desirable or possible to sample the reference location each time that dredged material is to be tested. Values from the homogeneous reference location collected over a period of time are used to develop decision guidance values which are compared to those obtained from benthic toxicity and bioaccumulation tests of the material to be dredged.

Periodic Reference Area Approach: This approach could, theoretically, be used when it is not desirable or possible to sample the heterogeneous reference locations each time that dredged material is to be tested. Values from heterogeneous reference locations collected over a period of time are used to develop decision guidance values which are compared to those obtained from benthic toxicity and bioaccumulation tests of the material to be dredged.

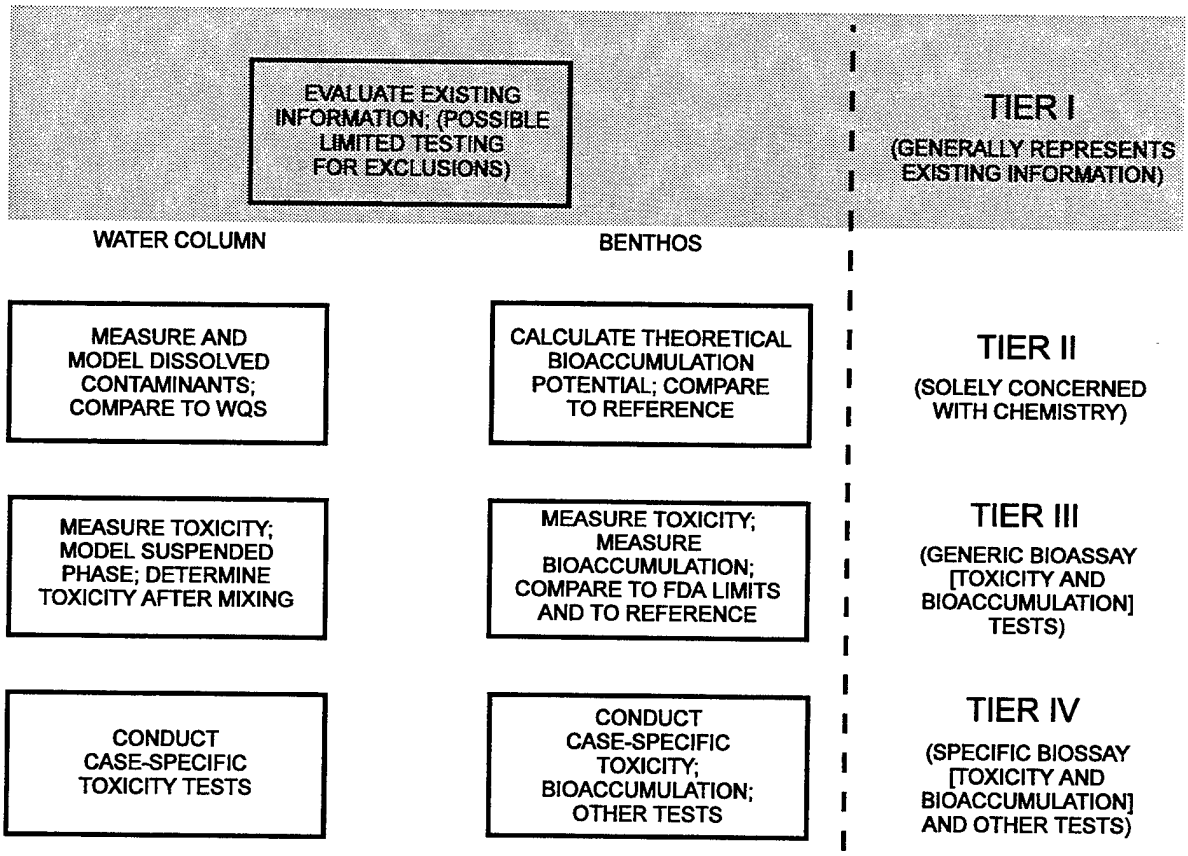
Appendix D, Statistical Methods, provides guidance for conducting statistical comparisons for the reference point and reference area approaches. It does not provide guidance for the use of either of the "periodic" approaches.

3.2.2 Reference Sediment Sampling Plan

The importance of thoughtful selection of the reference sampling approach cannot be overemphasized. To ensure that an appropriate approach is used, information gathered during the site specification process or other studies should be consulted for both the disposal and the reference sites. In some instances there are differences in the statistical methods used in comparing results from the various reference sampling methods to those obtained from the dredged material being

evaluated. There may also be differences in costs among the approaches. Prior to selecting an approach, it is imperative that Appendix D be consulted to determine which approach best fits specific concerns and conditions, including feasibility, technical validity, and cost.

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 (Section 8). The implementation of such a plan is equally essential for dredged material, control sediment, and reference sediment.


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)

4.0 TIER I EVALUATION

One of the purposes of Tier I is to determine whether factual determinations 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 monitoring data and testing for both the dredged material excavation site and the proposed disposal site. Only limited testing, to determine the applicability of exclusions, may be necessary in this tier.

If the information set compiled in Tier I is adequate to meet the exclusions or is complete and comparable to that which would satisfy Tier II, III, or IV, as appropriate, factual determinations can be made without proceeding into the higher tiers (Figure 3-1). For an evaluation to be completed within Tier I, the burden of evidence of the collected information must be adequate to make factual determinations.

The initial focus of the Tier I evaluation is on information relevant to Sections 230.60 (a), (b), (c), and (d) of the Guidelines and the potential for contaminant-associated impacts upon discharge. These four sections of the Guidelines fully define the exclusions from testing, which are summarized below.

If an evaluation of the dredging site indicates that the dredged material is not a "carrier of contaminants", testing may not be necessary. Such situations are most likely to arise if: the dredged material is composed primarily of sand, gravel and/or inert materials; the sediments are from locations far removed from sources of contaminants; the sediments are from depths deposited in preindustrial times and not exposed to modern sources of pollution. However, potential impacts from natural mineral deposits must also be considered.

Testing may also not be necessary "where the discharge site is adjacent to the excavation site and subject to the same sources of contaminants, and materials at the two sites are substantially similar "(Section 230.60 (c)). However, some physical and chemical testing may be necessary to confirm that the two sites are "substantially similar". The rationale behind this exclusion from testing is that when 1) the discharge and excavation sites are adjacent, 2) the concentration of contaminants in the two sites are not substantially different, and 3) the geochemical environments are similar, then the bioavailability of contaminants at the two sites are likely to be similar. This exclusion can apply even if the dredged material is a carrier of contaminants, providing that "dissolved materials and suspended particulates can be controlled to prevent carrying pollutants to less contaminated areas".

Section 230.60 (d) states that testing may not be necessary with material likely to be a carrier of contaminants if constraints acceptable to the USACE District Engineer and EPA Regional Administrator

are available to "reduce contamination to acceptable levels within the disposal site and to prevent contaminants from being transported beyond the boundaries of the disposal site". Such constraints may involve technologies such as capping and underwater containment. Design and monitoring requirements for such constraints should be determined by the Regional Administrator and District Engineer on a case-by-case basis.

If the exclusionary criteria are satisfied, factual determinations for the dredged material can be made and no further evaluation is necessary. If the exclusionary criteria are not met, the material is evaluated based on all existing information. This information should include chemical information and, if appropriate, existing data on the toxicity and bioaccumulation potential of the dredged material and of the reference sediment. The information must be sufficient to determine if water quality standards are met and, if appropriate, whether 1% of the LC_{50} or EC_{50} of each tested species will or will not be exceeded in the water column following mixing. If adequate information is not available for a Tier I evaluation, the process moves to Tier II.

Even if factual determinations cannot be made on the basis of Tier I information, the information collected can be put to use in later tier analyses. Another purpose of Tier I is to identify the contaminants of concern (if any) in the 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 tiers only until a factual determination is reached. Rigorous information collection and assessment in Tier I inevitably saves time and resources in making final determinations.

Annual or episodic dredging, undertaken to maintain existing navigation improvements, may warrant a periodic Tier I reevaluation. The general recommendation of EPA and USACE is that the interval between reevaluation of Tier I data for these projects not exceed three years or the dredging cycle, whichever is longest. If there is reason to believe that conditions have changed, then the time interval for reevaluation may be less than three years. As a minimum, this reevaluation should include a technical reassessment of all new and previously evaluated physical, chemical and biological data, changes in sediment composition or deposition (e.g., industrial development in the watershed), improvements in analytical methods and contaminant detectability, quality assurance considerations and any regulatory changes.

4.1 Compilation of Existing Information

The potential for contaminants to have been introduced to the dredged material, evaluated with consideration of the physical nature of the dredged material, and the proposed disposal site, allows case-by-case determinations of whether the proposed discharge of dredged material may result in contamination, bioaccumulation or toxicity above reference levels. Section 230.60 (b) of the Guidelines lists a number of factors which should be considered when evaluating the potential for contamination at the dredging (i.e., extraction) site. These factors represent sources of contamination, pathways of contaminant transport, and naturally occurring substances which may be harmful to aquatic biota:

- urban and agricultural runoff
- sewer overflows/bypassing
- industrial and municipal wastewater discharges
- previous dredged or fill discharges
- landfill leachate/groundwater discharge
- spills of oil or chemicals
- releases from Superfund and other hazardous waste sites
- illegal discharges
- air deposition
- biological production (detritus)
- mineral deposits.

The information gathering phase of Tier I evaluations has to be as complete as is reasonably possible, including existing information from all reasonably available sources. This will increase the likelihood that determinations concerning the impact of dredged material may be made at initial tiers. Sources of available information include the following, without limitation:

- Results of prior physical, chemical, and biological tests and monitoring of the material proposed to be disposed.
- Information describing the source of the material to be disposed which would be relevant to the identification of potential contaminants of concern.
- Existing data contained in files of agencies such as EPA or USACE or 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
- Agencies with contaminant or related information, for instance, Fish and Wildlife Service (FWS), National Oceanic and Atmospheric Administration (NOAA), regional planning commissions, state resource/survey agencies
- CWA 404(b)(1) evaluations
- Pertinent and applicable research reports
- MPRSA 103 evaluations
- Port and marina authorities
- Colleges/Universities
- Records of State agencies, (e.g., environmental, water survey, transportation, health)
- Superfund sites, hazardous waste sites
- Published scientific literature.

Sources may contribute differing types and quantities of contaminants to sediments. For example, a matrix of potential correlations between industrial sources and specific contaminants is provided in Table 4-1. This matrix is, however, not all inclusive and makes no accounting for current pollution control practices.

There are also a number of factors which influence the pathways between contaminant sources and the dredging and disposal sites, including:

- bathymetry
- water current patterns
- tributary flows
- watershed hydrology and land uses
- sediment and soil types
- sediment deposition rates.

More detailed site-specific guidance for reaching administrative decisions concerning the impact of a dredged material discharge may be developed by particular EPA Regions and USACE Districts by considering available scientific information and locally important concerns. In evaluating the likelihood

Table 4-1. Industries Associated with Sediment Contaminants. Data derived from Eckenfelder (1980), EPA (1987a), Merck (1989), WDNR/USGS (1992), EPA (1987b), NOAA (1991). Table developed by U.S. EPA Region 5, Water Division.

CONTAMINANTS / INDUSTRIES	Aluminum	Ammunitions	Anti-Fouling Paints	Automobile	Batteries	Chemical Manufacturing	Commercial Farming	Corrosion Metallurgy	Dairy	Detergents/Surfactants	Dye	Electrical	Explosives	Flat Glass	Fruits and Vegetables	Leather/Tanning	Meat Products	Metal Finishing Refining	Metallurgical Processes	Nitric Acid Manufacturing	Oxide Manufacturing	Perfume	Pesticides/Fertilizers	Petroleum Refining	Phosphate Mining	Phosphorus	Photographic	Pigments/Inks	Plastics	Printing Plates	Pulp and Paper Mills	Rubber	Steam Power	Steel/Iron	Sulfuric Acid	Textiles	Utilities	Valuable Mineral Mining	Waste Water Treatment Plants	Potential Nonpoint Sources	Boat Manufacturing/Boat Repair	Boat Refueling
Acenaphthene											●												●																		
Aldrin							●	●															●																		
Ammonia						●	●		●				●		●		●			●			●	●			●							●		●			●			
Aniline											●			●				●				●						●							●							
Arsenic				●														●	●							●	●					●	●		●						
Benzo(a)anthracene																		●																					●		
Benzo(a)pyrene				●														●																					●	●	
Cadmium	●			●		●						●						●																		●			●	●	●
Chlordane																																							●	●	
Chlorpyrifos																																							●		
Chromium	●			●		●		●										●			●																			
Copper		●	●	●				●				●	●			●		●			●						●						●					●	●	●	
Cyanide								●										●			●												●					●	●	●	
DDE																																		●								
DDT							●																																	●		
Dieldrin							●																●																	●		
Endrin																							●																			
Ethyl Parathion																							●																			
Fluoranthene																																										
Heptachlor																							●																	●	●	●
HCB																							●																			
HCBD																							●																			
HCCPD					●																																					
Lead			●		●			●				●						●						●				●	●				●	●			●				
Mercury			●			●						●						●										●	●								●	●	●		
2-Methylnaphthalene						●						●																●			●				●		●	●	●		
Nickel	●							●				●						●	●																		●	●			
Oil and Grease				●		●						●						●	●												●		●				●	●	●	●
Organotin/Tin			●																																		●	●			
PCBs				●								●																												●		
Phenanthrene																																		●				●			
Phosphorus		●					●		●	●					●								●		●	●	●			●			●		●		●	●		●
Pyrene																																		●		●		●	●		
Selenium						●						●		●										●			●	●									●	●	●	●	●
TCDD																								●			●	●			●					●			●		
TCDF																								●							●										
Toxaphene						●	●																●	●							●										
Zinc		●		●				●		●		●						●	●		●									●		●		●		●	●	●	●	

that discharge of a dredged material may cause contaminant associated impacts, concern decreases with the increase of factors such as:

- isolation of the dredging operation from known existing and historical sources of contamination
- time since historical sources of contamination 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 impacts 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
- included in continuing input from existing sources
- included in historical sources.

These and other considerations are complexly interrelated; i.e., the acceptable degree of isolation from sources of contamination 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 must be compatible with the Guidelines.

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 make a factual determination. A critical prerequisite to generating this information and one which is crucial to the success of the testing program is deciding, on a case-by-case basis, which contaminants are of concern, particularly for 401 certification, in the 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. Contaminants of concern are not restricted to compounds

which inhibit organisms but also those which promote undesirable organisms or growth (e.g., nutrients such as phosphorous - Nakaniski et al., 1986). However note that in at least some cases nutrient releases may be minimal and of no environmental concern (e.g., Tavolaro and Mansky, 1985).

4.2.1 Microbial Contamination

As noted in Section 2.2, this manual only addresses microbiological concerns to the extent that they address State 401 certification requirements. To this end, major areas of concern and pertinent sources of information addressing these and other relevant microbiological issues are provided below.

If sediments are suspected to have high levels of microbial contamination and dredging or disposal sites are close to shellfish beds, swimming beaches or drinking water intakes, then microbial sediment analyses may be required. Useful references include: EPA (1978); Gerba et al. (1979); Dutka et al. (1988) and Helmer et al. (1991). Appropriate state health and water quality agencies should be consulted for guidance and appropriate methods for measuring microbial contamination.

There are three major areas of concern for microbiological contamination and effects related to dredged sediments: (1) contamination of harvestable shellfish (e.g., Hood et al., 1983; Bruckhardt et al., 1992; Martinez-Manzanares et al., 1992); (2) body contact, generally related to swimming beaches (e.g., Fleisher, 1991; Helmer et al., 1991) ; (3) contamination of drinking water (e.g., Geldreich, 1991; Helmer et al., 1991). As noted in the Guidelines (e.g., 230.21, Suspended Particulates, and elsewhere), the ultimate concern is that "...pathogens and viruses...may be biologically available".

Sediments generally contain higher concentrations of indicators of fecal contamination and pathogens, such as *Salmonella* and viruses, than occur in the water column (e.g., Chen et al., 1979; Gerba et al., 1979; LaBelle et al., 1980). Further, these microorganisms survive longer in the sediments than in the water column (e.g., DeFlora et al., 1975; Smith et al., 1978; Borrego et al., 1983; Rao et al., 1984). Sediments have been shown to be a source of microorganisms released to the water column (e.g., VanDonsel and Geldreich, 1971; Shiharis et al., 1987; Hardina and Fujioka, 1991). More specifically, dredging and disposal have been shown to release these microorganisms (e.g., Grimes, 1975; Babinchak et al., 1977).

4.2.2 Chemical Contamination

Nationally, it is difficult to specify a single set of contaminants that adequately addresses all environmental concerns. However, regions may develop their own general contaminants of concern list for routine permitting purposes. In some dredged materials, there may be no contaminants of concern. Different disposal operations may have their own set of contaminants of environmental concern that should be adequately evaluated for each operation.

Identifying specific contaminants that are of concern in a particular dredged material is dependent on the information collected for Tier I. In some instances, it may be sufficient to perform confirmatory analyses for specific contaminants of concern identified in Tier I. 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 bulk sediment chemistry analysis conducted according to the guidance in Section 9.3 may be appropriate and, in fact, would be necessary to conduct the Tier II water quality screen and the theoretical bioaccumulation potential determination. Contaminants always of interest, if present, are those for which there are FDA limits or state fish advisories and where WQS exceedances exist. Other contaminants that should be included are those that might reasonably be expected to cause an unacceptable adverse impact if the dredged material is discharged.

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, 9.4, and 9.5:

- presence in the dredged material
- presence in the dredged material relative to the concentration in the reference sediment
- 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 solu-

bility. The octanol/water partition coefficient (K_{ow}, log K_{ow}, 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 K_{ow} of about 6.00. At hydrophobicities greater than about log K_{ow} = 6.00, bioaccumulation factors tend not to 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 (although some contaminants degrade to other contaminants which do bioaccumulate). 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 oxidative metabolism and subsequent conjugation with polar molecules. 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 tend to 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 can correlate with higher bioaccumulation unless the molecule is easily metabolized by an organism.

4.3 Tier I Conclusions

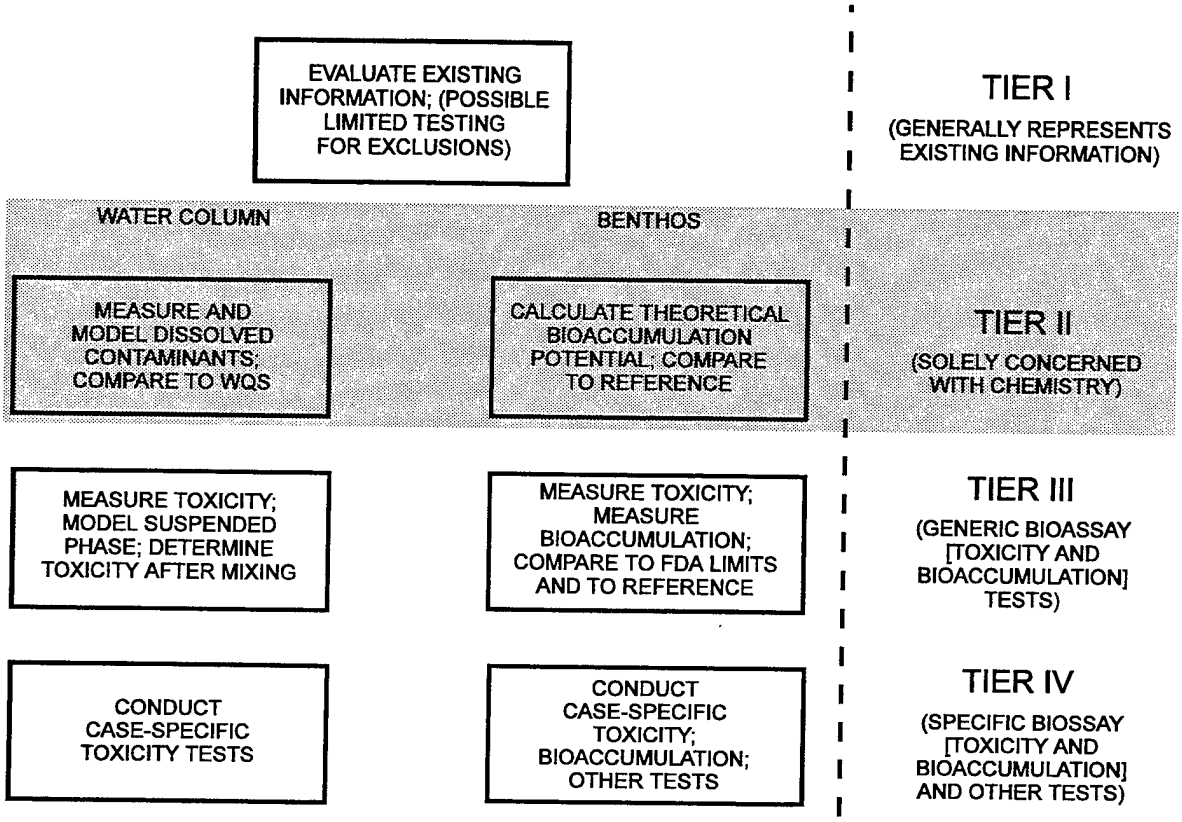
After consideration of all available information, one of the following conclusions is reached (Figure 3-1):

- Existing information does not provide a sufficient basis for making factual determinations. In this case, further evaluation in higher tiers is appropriate.

- Existing information provides a sufficient basis for making factual determinations. In this case, one of the following decisions is reached (Figure 3-1):

  - The material meets the exclusion criteria.

  - The material does not meet the exclusion criteria but information concerning the potential impact of the material is sufficient to make factual determinations.


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)

5.0 TIER II EVALUATION

Tier II provides useful information through screening tools, but not all possible determinations can be reached at this tier. It consists of evaluation of State water quality standard (WQS) compliance using a numerical mixing model of the disposal site conditions (Figure 3-2 and Appendix C) and an evaluation of the potential for benthic impact using calculations of theoretical bioaccumulation potential (TBP) (Figure 3-3 and Section 10.2).

Tier II is ultimately expected to provide a reliable, rapid screen to determine potential dredged material contaminant effects. The dredged material discharge must meet applicable WQS for all contaminants of concern outside the mixing zone. Water column impact must also be evaluated by toxicity testing in Tier III (Figure 3-2) when there are contaminants of concern for which applicable WQS are not available or where interactive effects are of concern.

When national sediment quality criteria (SQC) are proposed and finalized they are expected to provide a basis for State sediment quality standards (SQS). State SQS will be incorporated into Tier II benthic impact evaluations. The incorporation of these standards into Tier II will be implemented in this testing manual and regional manuals as appropriate.

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 TBP for a test organism by factoring the concentration of the nonpolar organic chemical(s), the total organic carbon in the sediment, and the percent lipid concentration in the organism. This calculation predicts the magnitude of bioaccumulation likely to be associated with nonpolar organic contaminants in the dredged material. Additional guidance for identifying potential bioaccumulating contaminants is provided by EPA (1994a).

5.1 Water Column Impact

Program experience (primarily in marine, near coastal and estuarine waters) has shown that in most cases the existing data are sufficient to make water column determinations. However, Tier I evaluation may show that the existing information is insufficient to make a determination. If a WQS determination cannot be made in Tier I, Tier II evaluation is necessary to determine whether the discharge complies with 230.10(b)(1) (Figure 3-2). The discharge of dredged material cannot cause the WQS to be exceeded outside the mixing zone unless the State provides a variance to the standard.

There are two approaches for the Tier II water column evaluation for WQS compliance. One approach is to use the numerical models provided in Appendix C as a screen, assuming that all of the contaminants in the dredged material are released into the water column during the disposal process. The other approach applies the same model with results from chemical analysis of the elutriate test.

5.1.1 Screen Relative To WQS

The assumption that all of the contaminants in the dredged material are completely released into the water column during the discharge operation is conservative because, in virtually all cases, most of the contaminants remain within the dredged material. If the numerical model (Appendix C) predicts that the concentrations of all contaminants of concern after consideration of mixing are less than the available, applicable WQS, the dredged material complies with WQS. If the screen/model, as applied indicates that the WQS is exceeded, the elutriate analysis approach (Section 5.1.2) should be employed.

5.1.2 Elutriate Analysis Relative To WQS

For an elutriate analysis, the numerical mixing model (Appendix C) 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 provided in Section 9.4.2. The model is, in effect, using data that more accurately represent the contaminant concentrations that will be present in the water column after consideration of mixing. If the numerical model (Appendix C) predicts that the concentration of all contaminants of concern at the edge of the mixing zone is less than the available, applicable WQS, the dredged material complies with WQS. Otherwise, it does not.

5.2 Benthic Impact

The currently available Tier II procedure for evaluating potential benthic impact consists of evaluating the TBP, calculated according to the guidance in Section 10.2. A comparison is made between the TBP calculated for the nonpolar organic contaminants of concern in dredged material and for the same constituents in the reference sediment. 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, further evaluation has to take place in Tier III.

Even if the dredged material contains other contaminants of concern than nonpolar organic contaminants, 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 actual testing (Tiers III and/or IV). Additionally, the calculation may eliminate the need for further evaluation of nonpolar organics and thereby reduce efforts in higher tiers.

5.3 Tier II Conclusions

One of two possible conclusions is reached regarding the potential water column impact of the proposed dredged material:

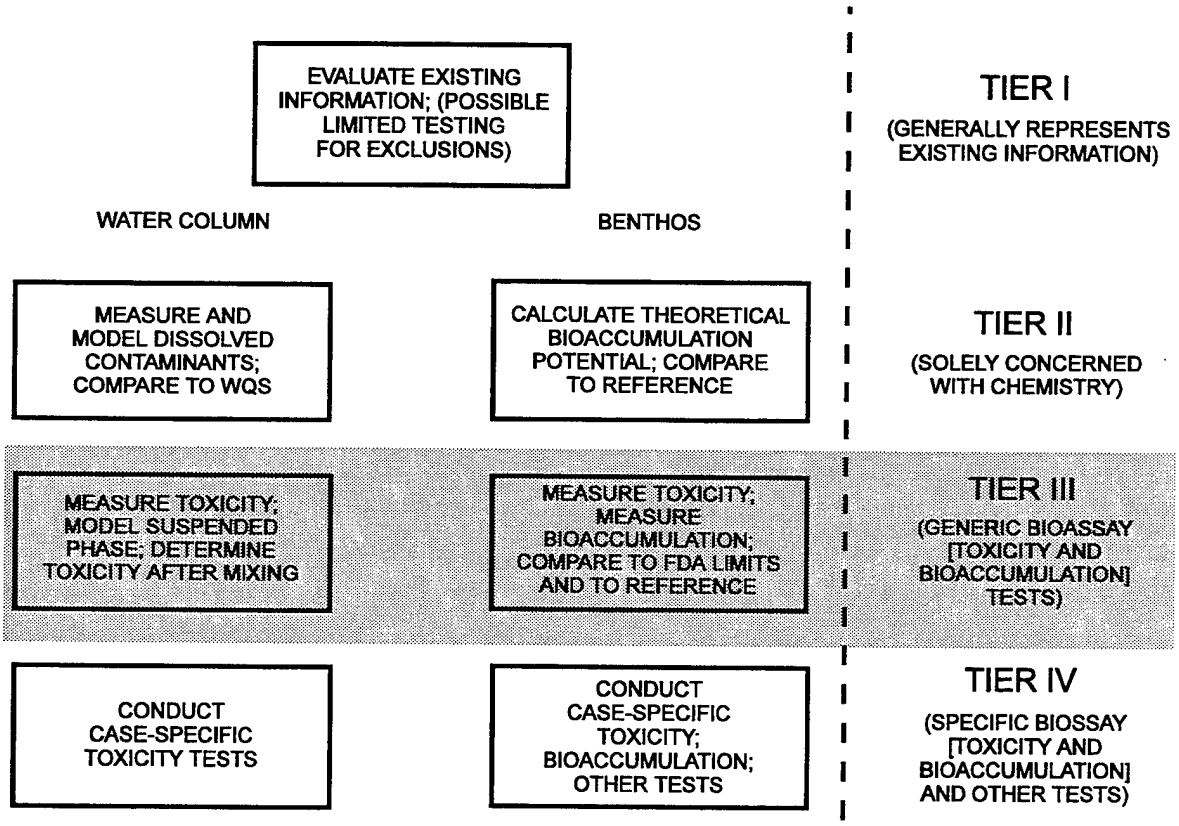
- The available WQS requirements are met. Further information on water column toxicity must be evaluated in Tier III when there are contaminants of concern for which applicable WQS are not available or where interactive effects are of concern.

- Concentrations of one or more of the dissolved contaminants of concern, after allowance for mixing, exceed available WQS beyond the boundaries of the mixing zone. In this case, the proposed discharge of dredged material does not comply with WQS.

For nonpolar organics, one of the following conclusions is reached based on comparison between the TBP for the dredged material and for the same contaminants in the reference sediment:

- 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 is predicted not to result in benthic bioaccumulation of the measured non-polar organic compounds. However, further evaluation of biological effects in Tier III is necessary to furnish information to make determinations under the Guidelines.

- The TBP for the nonpolar organic contaminants of concern in the dredged material exceeds the TBP for the reference sediment. In this case, the information is not sufficient to predict whether the dredged material will result in benthic bioaccumulation of the measured non-polar organic compounds, and further evaluation of bioaccumulation in Tier III is necessary to furnish information to make determinations under the Guidelines.


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)

6.0 TIER III EVALUATION

Tier III testing assesses the impact of contaminants in the dredged material on appropriately sensitive and benchmark organisms to determine if there is the potential for an unacceptable (toxicity or bioaccumulation) impact at the disposal site. Lists of candidate test species (Sections 11 and 12: Tables 11-1 through 12-1) include consideration of: (1) appropriate sensitivity such that testing should not occur with insensitive organisms; (2) allowing appropriate Regional flexibility based on the list provided in this manual or the approved regional implementation manual; (3) providing some benchmark species for comparing (where appropriate) the sensitivity of regional species not widely used for such testing.

The Tier III assessment methods are bioassays (toxicity and bioaccumulation tests) (Figures 3-1 through 3-3). Generic guidance provided in this manual may have to be modified for specific species. Where possible and appropriate, organisms representative of the water column and benthic biota and conditions at the disposal site or the appropriate reference area should be used. Also, exposure routes must be appropriate (e.g., benthic test species must be truly benthic, that is, living on or in the sediment).

Presently, Tier III toxicity tests primarily use lethality as the endpoint. Chronic/sublethal tests for sediments are under development; none are considered to be currently suitable for wide-spread national use and hence are not included in this manual although regional use is allowed (cf. Section 11.2.3). New, appropriate benthic and water column tests, including sediment chronic/sublethal tests, will be included in future revisions of this manual as appropriate.

The recommended procedures for water-column toxicity tests (Figure 3-2) use appropriate sensitive 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 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 (Kay, 1984).

Tier III information is usually sufficient for making factual determinations. Only in unusual cases is further information on toxicity or bioaccumulation (or both) necessary to make determinations under the Guidelines.

6.1 Water Column Toxicity Tests

Tier III (Figure 3-2) considers the effects on water column organisms, after allowance for mixing, of dissolved contaminants plus those associated with suspended particulates. The toxicity and mixing data results are generated as described in Section 11.1.

After considering the tests and considering mixing, one of the following conclusions is reached:

- If the 100% dredged material elutriate toxicity is not statistically higher than the dilution water (see Section 8.0, Table 8-1), the dredged material is not predicted to be acutely toxic to water column organisms.

- The concentration of dissolved plus suspended contaminants, after allowance for mixing, does not exceed 0.01 of the toxic (LC_{50} or EC_{50}) concentration beyond the boundaries of the mixing zone. Therefore the dredged material is predicted not to be acutely toxic to water column organisms. However, benthic impact has to be considered. If the information warrants, it is acceptable to determine water column effects at Tier III and benthic effects at another tier.

- The concentration of dissolved plus suspended contaminants, after allowance for mixing, exceeds 0.01 of the toxic (LC_{50} or EC_{50}) concentration beyond the boundaries of the mixing zone. Therefore, the dredged material is predicted to be acutely toxic to water column organisms.

6.2 Benthic Toxicity Tests

Evaluation of benthic (i.e., sediment) toxicity tests in Tier III (Figure 3-3) is based on data generated according to the guidance in Section 11.2. Dredged material is predicted to be acutely toxic to benthic organisms when mean test organism mortality:

- is statistically greater than in the reference sediment, and

- exceeds mortality (or other appropriate end point) in the reference sediment by at least 10% (the 10% value should be used unless a different value has been developed for specific test species and end-points for regulatory use, and is technically defensible; e.g.,

a 20% value for lethality can be used for the amphipods *Ampelisca abdita, Rhepoxynius abronius* and *Eohaustorius estuarius* (Swartz et al., 1985; Mearns et al., 1986; SAIC, 1992a,b)).

However, even if there is a certain level of toxicity (e.g., marginal mortalities for a single non-benchmark species), the preponderance of evidence could suggest that the sediment is not acutely toxic to benthic organisms. Acute toxicity testing of contaminants in the dredged material in Tier III will result in one of the following possible conclusions:

- Mortality (or other appropriate endpoint) in the dredged material is not statistically greater than in the reference sediment, or does not exceed mortality (or other appropriate endpoint) in the reference sediment by at least 10%. Therefore, the dredged material is predicted not to be acutely toxic to benthic organisms. However, bioaccumulation of contaminants also has to be considered. If the information warrants, it is acceptable to determine benthic toxicity at Tier III and bioaccumulation at another tier.

- Mortality (or other appropriate endpoint) in the dredged material is statistically greater than in the reference sediment and exceeds mortality (or other appropriate endpoint) in the reference sediment by at least 10%. In this case, the dredged material is predicted to be acutely toxic to benthic organisms.

6.3 Benthic Bioaccumulation

Body burdens of chemicals are of concern for both ecological and human health reasons. The Tier III benthic bioaccumulation tests (Section 12.1) are conducted for a subset of the contaminant of concern list based on the contaminant bioaccumulation properties discussed in Sections 4.2 and 10.2. These tests provide for the determination of bioavailability through 28-day exposure tests. For purposes of comparison with an action or tolerance level such as from Food and Drug Administration (FDA) as described below (or when conducting a Tier IV risk assessment), the duration of a bioaccumulation test should be sufficient for organisms to reach steady-state tissue residues for all compounds. However, the time to reach or approach steady-state varies among different compounds and, to a lesser extent, among species. Test designs that assure that steady-state has been attained require a large number of samples and substantial expense. As a cost-effective compromise, it is recommended that a 28 day exposure be used for the "standard" bedded sediment bioaccumulation test for neutral organics and metals.

Where it is desirable to know the steady-state concentration of neutral organic compounds as, for example, comparison to an FDA action level, fish advisory, or similar numerical values, the following procedure is recommended. The log Kow of the neutral organic compound of concern should be determined from Section 9.5.1 (Table 9-5). This should be compared with the log Kow in Figure 6-1 and will indicate the proportion of steady-state concentration (Css) expected in 28 days. This will allow estimation of the steady-state value from the 28-day laboratory exposure data through the use of a steady-state correction factor. The correction factor is the reciprocal of the decimal fraction indicating the proportion of Css expected in 28 days.

Bioaccumulation of most compounds, if it occurs, will be detectable after the 28-day exposure period, even though steady state may not have been reached. Thus, Tier III bioaccumulation tests provide useful information about the potential for bioaccumulation (i.e., bioavailability), even when steady-state tissue residues are not determined, e.g. when comparing to a reference sediment.

Concentrations of contaminants of concern in tissues of benthic organisms following dredged material exposure are compared to applicable Food and Drug Administration (FDA) Action or Tolerance 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. The FDA levels (Table 6-1) are based on human-health as well as economic considerations (21 CFR 109 and 509), but do not indicate the potential for environmental impact on the contaminated organisms or the potential for biomagnification. Because contamination of food in excess of FDA levels is considered a threat to human health, EPA and USACE consider concentrations in excess of such levels in any test species to be predictive of benthic bioaccumulation of contaminants. This guidance applies even though the test species may not be a typical human food item partly because certain contaminants can be transferred through aquatic food webs, but mainly because uptake to FDA levels in relatively short term tests with one species may indicate the potential for accumulation in other species.

Based on tissue comparisons with FDA levels, one of the following conclusions is reached:

- Tissue concentrations of one or more contaminants are not statistically less than the FDA levels. Therefore, the dredged material is predicted to result in benthic bioaccumulation of contaminants.

- Tissue concentrations of all contaminants either are statistically less than FDA levels or there are no FDA levels for the contaminants. In this case, the information is insufficient to reach a conclusion with respect to benthic bioaccumulation of contaminants. The

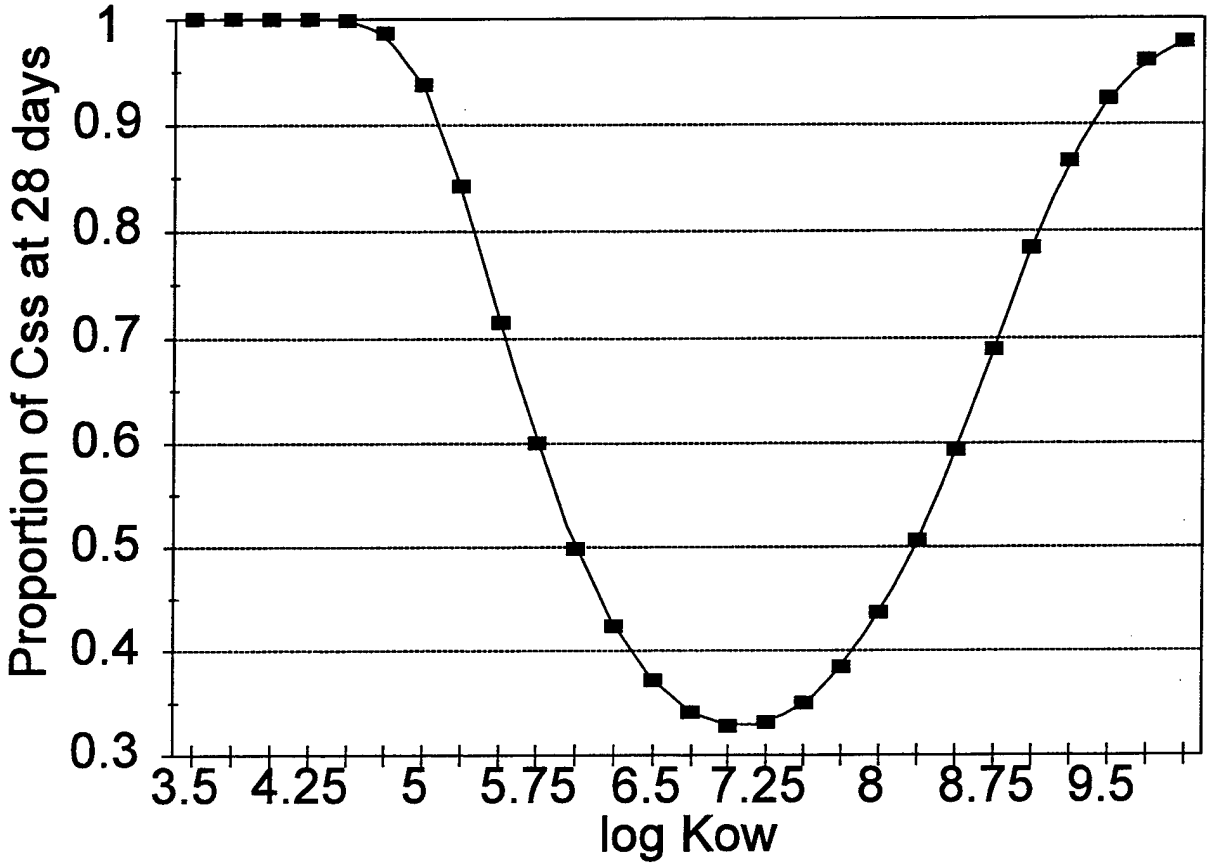


Figure 6-1. Expected proportion of steady-state concentration (Css) of neutral organic compounds reached in 28-day laboratory exposures. The proportion is a function of the log Kow of the compound of interest. Consult Section 9.5.1 (Table 9-5) for appropriate log Kow values. Figure adapted from McFarland (1994).

Table 6-1. Food and Drug Administration (FDA) Action Levels for Poisonous and Deleterious Substances in Fish and Shellfish for Human Food.[a]

Substance	Action Level[b]
Metals	
Methyl Mercury	1.0 ppm
Pesticides	
Chlordane	0.3 ppm
Chlordecone (Kepone)	0.3 ppm
DDT + DDE	5.0 ppm
Dieldrin + Aldrin	0.3 ppm
Heptachlor + Heptachlor Epoxide	0.3 ppm
Mirex	0.1 ppm
Industrial Chemicals	
PCBs[c]	(2.0 ppm)

[a] Action levels are established, revised, and revoked through notices published in the Federal Register. It is the responsibility of the users of the list to keep up to date on any amendments to this list. For further information on current action levels, users may contact the Food and Drug Administration, Center for Food Safety and Applied Nutrition, Industry Programs Branch [HFF-326, 200 C Street, S.W., Washington, DC 10204; (202) 205-5251].

[b] Action levels are reported in wet weight.

[c] There is no FDA action level for PCBs as a tolerance level has now been established (21 CFR part 109.30), which is equal to the previous action level.

dredged material needs to be further evaluated in Tier III as described below for bioaccumulation potential to furnish information to make determinations under the Guidelines.

Tissue contaminant concentrations following exposure to dredged material which are statistically less than FDA levels, or for which there are no such levels, are compared to tissue contaminant concentrations for 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; therefore, the dredged material is predicted not to result in benthic bioaccumulation of contaminants. However, benthic toxicity effects also have 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, the final conclusion regarding benthic bioaccumulation of contaminants would be based upon technical evaluations that emphasize the various factors deemed appropriate in a particular region (see last paragraph in this section). Additional testing (Tier IV) may be required.

One other possibility exists: tissue concentrations are above FDA limits but are not statistically different from the reference (or disposal) site. This situation represents an exceptional case which can only be dealt with at the regional level.

The above comparisons to FDA values address human health concerns, and follow from EPA/USACE (1991). Other approaches which should be considered in addition to the use of FDA values include comparisons to state fish advisories, cancer and non-cancer risk models, existing ambient fish concentration data. State fish advisories exist for the following chemicals for which EPA risk-based screening values are being developed: (carcinogens) chlordane, DDT, dieldrin, hexachlorobenzene, lindane, toxaphene, PAH, PCBs, 2,3,7,8-TCDD; (noncarcinogens) endosulfan, mirex, cadmium, mercury, selenium, endrin. Methods to calculate carcinogenic and non-carcinogenic health risks are summarized in EPA (1989a). "Computerized Risk and Bioaccumulation System", an expert system for PC computers, is available to predict tissue residues in sediment-dwelling shellfish and the associated excess cancer risk (Lee et al., 1990). Note that this program does not calculate risks associated with mobile invertebrates or fishes, and that it should be used only to supplement data derived from other methods.

Reference comparisons are made for the protection of aquatic life as well as human health because bioaccumulation is both undesirable and an indicator of bioavailability (Figure 3-3). It is recognized that residue effects information does not exist to fully interpret bioaccumulation data; the approach followed in this manual is the best presently available.

When the bioaccumulation of contaminants in dredged-material tests statistically exceeds that in reference-material tests, five factors should be assessed. Where available, regional guidance should be consulted regarding the relative importance of these factors:

- What is the toxicological importance of the contaminants (e.g., Do they biomagnify? Do they have effects at low concentrations?) whose bioaccumulation from the dredged material statistically exceeds that from the reference material?

- By what magnitude does bioaccumulation from the dredged material exceed bioaccumulation from the reference material?

- What is the propensity for the contaminants with statistically significant bioaccumulation to biomagnify within aquatic food webs (Kay, 1984)? Contaminants which biomagnify appear to be few in number but widespread, and include DDT, PCB, methylmercury and, possibly, dioxins and furans.

- What is the 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?

- For how many contaminants is bioaccumulation from the dredged material statistically greater than bioaccumulation from the reference material?

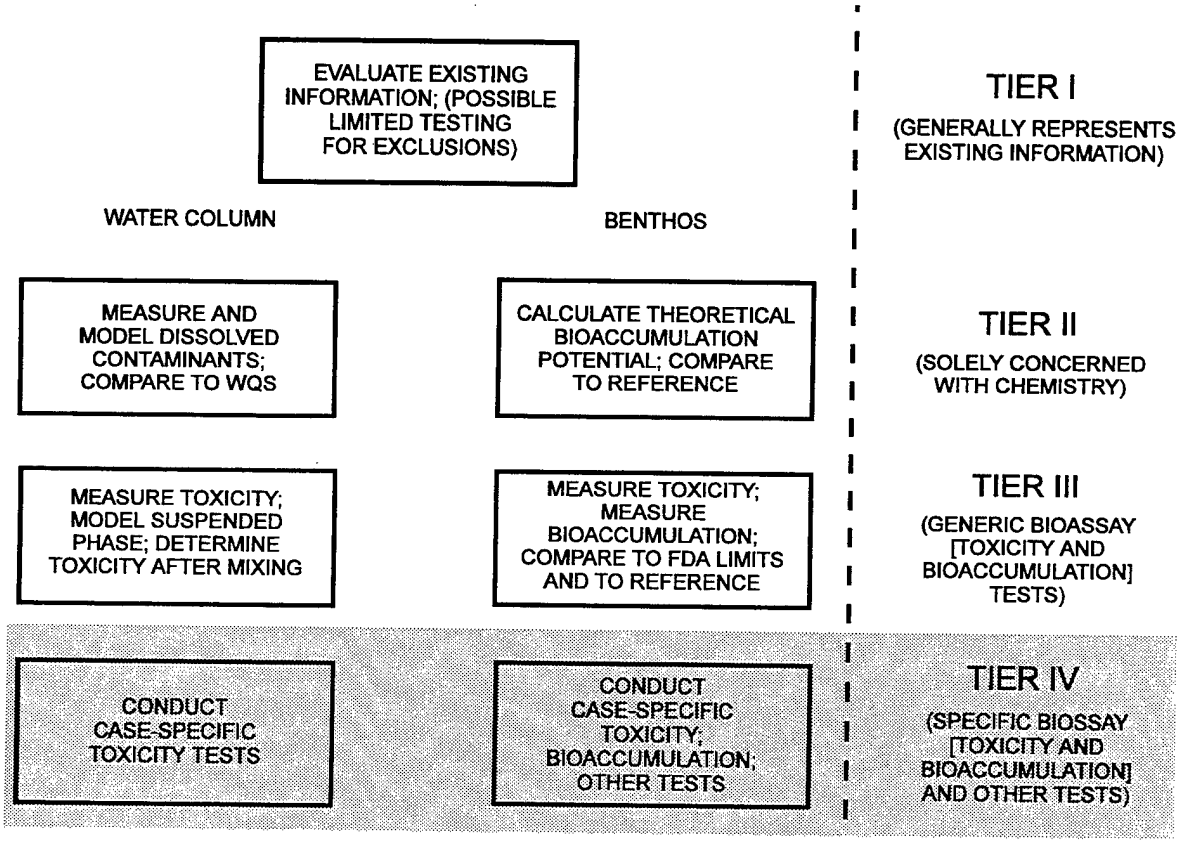
6.4 Tier III Conclusions

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

- Discharge of the dredged material is predicted not to result in above-reference toxicity or benthic bioaccumulation of contaminants.

- Discharge of the dredged material is predicted to result in above-reference toxicity or bioaccumulation of contaminants.

- Further information is needed to make factual determinations, specifically in Tier IV.


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)

7.0 TIER IV EVALUATION

Tier IV involves case-specific, state-of-the-art testing for toxicity and/or bioaccumulation and is to be used on a case-by-case basis only when lower tiered testing is judged to be insufficient to make complete factual determinations. Insufficient information for a determination may include: inability to reach a clear conclusion based on existing data; statistical differences are inconclusive; evidence is conflicting. Experience to date suggests that Tier IV should only be used in a very few cases. When methods are suitable for wide-spread national use, sediment chronic/sublethal testing will be part of Tier III. Until such time as sediment chronic/sublethal tests are approved for national use in Tier III, they should only be used in Tier IV. However, regional testing manuals may apply appropriate sediment chronic/sublethal tests in Tier III in advance of their inclusion in this national manual provided this is done with a benchmark species (Section 11.2.1) or *in addition to* the testing presently required in Tier III.

Tier IV tests may be conducted for water column evaluations (Figure 3-2) or benthic evaluations (Figure 3-3). In both cases, tests should be carefully selected to address the specific issues relevant to the case in question. Tier IV can further consider human and ecological health concerns, including risk assessment. Case-specific evaluative criteria for Tier IV tests must be:

- agreed upon by EPA and USACE and, where appropriate, the State
- adequate to make factual determinations.

7.1 Toxicity Tests

Tier IV toxicity tests (Figure 3-2) should measure end-points of clear ecological importance, for example survival, growth and reproduction. Differences from Tier III tests may include:

- longer duration of exposure
- different species
- different end-points
- exposure in the disposal site environs.

Toxicity determinations in this tier can involve laboratory or field testing or field assessments of resident benthic communities. Field assessments can be difficult to interpret but can yield valuable information on responses of resident organisms to in-place contaminants at the dredging site as compared to a disposal site or site environs as appropriate.

Toxicity identification evaluation procedures (e.g., Ankley et al., 1992a) can also be used in this tier. Such procedures can be applied to sediments when ammonia or hydrogen sulfide could be responsible for toxicity.

7.2 Benthic Bioaccumulation

Tier IV bioaccumulation tests (Figure 3-3) differ from Tier III tests in that steady-state tissue concentrations of contaminants of concern are always determined. Such determinations can be made by: longer laboratory exposures than used in Tier III, collecting tissue samples from the field (Section 12.2.2), or *in situ* exposures using transplanted organisms.

Tissue concentrations determined in Tier IV are subject to the same comparisons as in Tier III, specifically to FDA action limits, and to comparisons with organisms exposed to reference sediment. Conclusions possible from such comparisons and evaluative factors which should be assessed are detailed in Section 6.3 and can include risk assessments and no effects levels for aquatic life, rather than solely the first two comparisons.

Prediction of the movement of contaminants from sediment into and through pelagic food webs is technically challenging and should only be dealt with if a Tier IV evaluation is necessary. One approach is bioenergetic-based toxicokinetic modeling. These models have been successfully applied to marine (Connolly and Tonelli, 1985) and freshwater (Norstrom et al., 1976) fishes, theoretical food chains (Thomann, 1989), and more recently to sediment organisms (Boese et al., 1990). These models are very data intensive to apply on a chemical and site-specific basis. It is possible to use values determined through QSAR (EPA, 1994a), though the default values may substantially overestimate tissue residues in metabolizable compounds, such as PAH. Another general approach is to bracket likely concentrations of specific contaminants at different trophic levels based on an empirical model derived from a variety of marine food webs (Young, 1988).

PART III - SAMPLING AND ANALYSIS

8.0 SAMPLING

When testing is necessary, samples of dredged material, reference sediment, control sediment, organisms, and water will be needed for physical evaluations, chemical analysis, and for bioassay tests. This section provides general guidance for the development of a sampling plan including collection, handling and storage.

Sampling is the foundation upon which all testing rests but there are so many case-specific factors that influence sampling needs that detailed guidance of National scope is impractical. Some regions of the country have developed specific technical requirements and agency review/approvals of sampling and analysis plans. Regional guidance from local EPA and USACE offices should be sought for developing project-specific sampling plans as for information gathered at Tier I. The type of samples that may be required to complete the evaluations of Tiers II, III, and IV are outlined in Table 8-1. This manual provides general guidance on items of major importance to consider when designing a sampling plan. Additional guidance is provided by EPA (1995).

8.1 Preparation For Sampling

A well-designed sampling plan is essential when evaluating the potential impact of dredged material discharge upon the aquatic environment. Before any sampling is initiated, the sampling plan has to be tailored to meet clearly defined objectives for individual dredging operations. Factors such as the availability and content of historical data, the degree of sediment heterogeneity, the dredging depth, 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 (Appendix G; EPA, 1995) must be carefully considered. The magnitude of the dredging operation and its time and budgetary constraints should also be considered.

It is recommended that a written plan for sediment sampling and analyses be prepared and provided to the appropriate Federal and State agencies for coordination prior to sampling, where practicable. The Tier I evaluation would be a logical attachment to the sampling and analysis plan for agency review and comment. This coordination can reduce the chance of having to repeat costly procedures and can assist in keeping projects on schedule. An adequate amount of sediment and water should be collected to conduct planned evaluations and allow for any contingencies. Maximum allowable and recommended sample and organism holding times as well as the exigencies of resampling should be given careful consideration.

Table 8-1. Type of Samples Which May Be Required Following Tier I to Conduct Dredged-Material Evaluation Tests. Actual sampling requirements are project-specific and are determined during the development of the project plan. Sampling from the disposal site may also be conducted as necessary and appropriate, to verify the applicability of exclusion 230.60 (C) (see Sections 4.0 and 9.1.)

Tests	Water Samples			Sediment Samples			Biota Samples	
	Disposal Site	Dredging Site	Control[a]	Dredging Site	Reference Site	Control[a]	Dredging Site	Reference Site
Tier II								
Water column								
Screen	●[c]			●				
Elutriate	●[c]	●		●				
Tier II								
Benthic				●	●			
Tier III								
Water column	●[b]	●	●	●				
Tier III								
Benthic				●	●	●		
Tier IV								
Water column	●	●	●	●			●	●
Tier IV								
Benthic				●	●	●	●	●

[a]May or may not have to be field-collected.

[b]Dilution water for water column toxicity tests. Artificial or clean seawater or clean freshwater may also be used.

[c]Disposal site water is required for WQS comparison. Elutriate samples are prepared with dredging site water.

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 limit the accuracy and/or the usefulness of the study results.

The primary objective of sediment and water collection is to obtain samples to adequately and accurately characterize the dredging and reference area. Sample size should be large enough to attain the appropriate detection limits but small enough to be conveniently handled and transported within the requirements for all planned analyses. The quality of the information obtained through the testing process is impacted by the following four factors:

- collecting representative samples
- collecting an appropriate number of samples
- using appropriate sampling techniques
- protecting or preserving the samples until they are tested.

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

An important component of any field sampling program is a preproject meeting with all concerned personnel. Personnel involved may include management, field personnel, laboratory personnel, data management/analysis personnel, and representatives of regulatory agencies, the permit applicant, and the dredging company. To assure sampling quality, at least one individual familiar with the study area should be included in the preproject meeting. The purposes of the meeting include:

- defining the objectives of the sampling program
- ensuring communication among participating groups
- ensuring agreement on methods, QA/QC details and contingency plans.

The more explicitly the objectives of a testing program can be stated, the easier it will be to design an appropriate sampling plan. A complete sampling plan will result in a level of detail such that all sampling procedures and locations are clearly defined, sample volumes are clearly established, all logistical concerns are fully addressed, and target analytes are identified to class of compound.

8.2 Components Of A Sampling Plan

The following steps will help to ensure that all essential sampling plan information is provided:

- Review the plan for the proposed dredging operation, including the dimensions of the dredging area, the dredging depth(s), side-slopes, the volume of sediment for disposal, and the type of dredge equipment (e.g., clamshell, hydraulic) for determining composite sampling or delineating representative project segments.
- Evaluate the prior history and the existing database for the area, in particular, information gathered in Tier I. 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, storage, and (if applicable) field or shipboard analysis.
- Identify logistical considerations and safety precautions.

The subsections that follow discuss each of these steps and provide general guidance for their conduct. An essential step, preparation of a quality assurance/quality control (QA/QC) project plan, is discussed in detail in Appendix G and EPA (1995) and must be integral to the project. The QA/QC plan is essential to ensure that there will be sufficient and appropriate data of known and documented quality to make decisions with confidence and to defend those decisions. Properly prepared, a QA/QC plan expedites project coordination.

8.2.1 Review of Dredging Plan

A review of the plan for the dredging operation provides a basis for determining the sampling strategy. The volume of material to be dredged and the method of dredging are two important factors which will help to determine the number of samples required. The number of samples required is generally a judgement which considers the cost, resolution, and the risk of an incorrect decision regarding the volume of material to be dredged. Knowledge of the depth 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. Sampling should generally be to the project depth (including overdredging) unless the sediments are known to be vertically homogeneous.

8.2.2 Historical Data

All information relevant to the dredging site should be reviewed. Using pertinent available information to determine project segments and station locations within the dredging area is both cost and technically effective. If a review of historical data identifies possible sources of contamination, skewing the sampling effort toward these areas may be justified for thorough characterization of these areas, but can lead to an incomplete assessment of contamination in the whole area. In areas of unequally distributed contamination, the total sampling effort should be increased to ensure representative, but not necessarily equal, sampling of the entire site. Sediment sampling techniques are detailed in Mudroch and MacKnight (1991). The information gathered for the Tier I evaluation (discussed in Section 4.1) should be reviewed for assistance in designing the sampling plan, in particular the following:

- **Geotechnical and hydrodynamic data**

  The grain size, specific gravity, water or solids content, total organic carbon (TOC) and identification of sediment horizons are helpful in making operational decisions. Areas of high currents and high wave energy tend to have larger grain-sized sediments than do quieter areas. Many contaminants have a greater affinity for clay and silt than for sand. Horizontal and vertical gradients may exist within the sediment. Local groundwater quality and movement should be determined if groundwater is a potential source of contamination.

- **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. Existing data that do not meet all quality assurance/quality control (QA/QC) standards may still be useful if appropriate calibration and documentation are available; they are less useful if older methods with higher detection limits were used. Information from such studies might be helpful in identifying areas of contamination, but not in accurately assessing the degree of contamination.

- **Known distribution of contaminants**
  All evidence regarding contaminants within or near the dredging area, including spill data, may be an important consideration in identifying locations for sampling and/or determining sampling intensity.

- **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, currents, or ship traffic, the sediments are likely to be relatively homogeneous. Assuming that there is no major contaminant input, the sampling effort may be minimal. However, if there is information regarding possible contamination or heterogeneity is possible, a more extensive sampling effort may be indicated. New excavations of material unaffected by anthropogenic input may require less intensive sampling than 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. Areas of low hydrodynamic energy will be characterized by fine sediments that have a greater tendency to accumulate contaminants than do coarser-grained sediments. (However note that contaminants, if present in coarse-grained sediments, may be more bioavailable than if present in fine-grained sediments). Sediments in and downstream of heavily urbanized or industrialized areas are more likely to accumulate contaminants than sediments farther removed from direct contaminant input.

Many dredging operations can be subdivided into project segments (horizontal and/or vertical) which can be treated as separate management units. 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 the study objectives and test results, the dredged material from various project segments can be managed differently during dredging and disposal to limit environmental impact. When the sampling plan is developed, project segments can be designated, based on factors including but not limited to: historical data, sediment characteristics, geographical configuration, anticipated method of dredging, depth of cut, sampling- or dredging-equipment limitations, results of pilot studies, and known or suspected contaminant concentrations. Surface sediments might be considered separately from subsurface sediments at the same location if vertical stratification of contamination is expected or encountered. 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, which are likely to be relatively homogenous vertically. Vertical subdivisions smaller than about 1 m are usually impractical because dredge operators generally cannot reliably control excavation with any finer precision; vertical subdivisions should reflect the actual removal precision to be employed during the dredging operation. If analytical data and test results for two or more project segments prove to be similar, these segments may be treated as one larger segment when considering disposal options. If the analytical and test results demonstrate important differences between project segments, alternative disposal options may be necessary for portions of the total sediment volume.

Any established sampling program should be sufficiently flexible to allow changes based on field observations; however, any deviations from the sampling plan must be documented, along with the rationale for such deviations. Certain characteristics of the sediments, such as color or texture, can be an indication of patchiness. The greater the patchiness, the larger the number of samples that will be required to adequately characterize 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 Locations and Number of Samples

Generally a single sampling strategy will be adequate for most circumstances. However, in some cases, two sampling strategies may be required. For instance, when sampling involves both uncontaminated and highly contaminated sediments with interfaces between the two, a single sampling strategy may not be sufficient to adequately characterize these sediments, which will probably be treated differently.

The method of dredging, the volume of sediment to be removed, the areal extent of the dredging project, 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 should be determined for each operation on a case-by-case basis. Note that the following detailed discussion is not appropriate to all dredging operations. Sampling a number of small, isolated shoals is very different than sampling a large, contiguous open area.

Factors to Consider:

The following factors, many of which follow from information gathered in Tier I, should be among those considered in sampling station and pattern selection:

- objectives of the testing program
- bathymetry
- area of the dredging project
- accessibility
- flows (currents, tides)
- mixing (hydrology)
- sediment heterogeneity
- contaminant source locations
- land use activities
- available resources
- other physical characteristics.

Station Locations:

Station locations within the dredging area should include locations 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. Characteristics which help to define the representativeness of station(s) within a segment include:

- The distribution of sediments to be dredged is clearly defined.
- The project segment being sampled is clearly defined.
- The sampling locations are distributed appropriately within each project segment.
- Multiple samples should be collected 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.

Sample Replication:

Within a station, samples may be collected for replicate testing. For this manual, laboratory replicates are generally recommended as opposed to field replicates, depending on site-specific issues. The former (subsamples of a composite sample of the replicates) involves pseudo-replication compared to separate samples for each replicate, but is more appropriate for dredged material evaluations where sediments will

be homogenized by the dredging and discharge process. The latter involves true replication but is more appropriate for field investigations of the extent and degree (or not) of homogeneity of sediment toxicity.

Depth Considerations:

Sediment composition can vary vertically as well as horizontally. Samples should be collected over the entire dredging depth (including over-dredging), unless the sediments are known to be vertically homogeneous or there are adequate data to demonstrate that contamination does not extend throughout the depth to be excavated. Separate analyses of defined sediment horizons may be useful to determine the vertical distribution of contamination if warranted by the study objectives. A major consideration of vertical compositing is the anticipated depth of dredging. For example, even though sediments in a 1 m shoal may vary in composition, the material would be mixed as a result of the dredging process.

Sampling Bias:

Ideally, the composition of an area and the composition of the samples obtained from that area will be the same. However, in practice, there often are differences due to bias in the sampling program, including disproportionate intensity of sampling in different parts of the dredging area and equipment limitations.

In some cases, to minimize bias, it may be useful to develop a sampling grid for each project segment. The horizontal dimensions of each project segment may be subdivided into grid cells of equal size, which are numbered sequentially. Cells are then selected for sampling either randomly or in an stratified random manner. It can be important to collect more than the minimum number of samples required, especially in areas suspected of having high or highly variable contamination. In some cases, although additional costs and logistic considerations will apply, extra samples may be archived (for long time periods in the case of physical characterization or chemical analyses and for short time periods in the case of biological tests), should reexamination of particular project segment(s) be warranted.

In other cases, a sampling grid may not be desirable. This is particularly the case where dredging sites are not continuous open areas, but are rather a series of separate humps, bumps, reaches and pockets with varying depths and surface areas. In these latter cases, sample distribution is commonly biased with intent.

Level of Effort:

In some cases, it may be advisable to consider varying the level of sampling effort. Project segments suspected or known to be contaminated may 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, and level of concern can then be used as a factor when determining the number of samples within each project segment relative to other project segments.

Number of Samples:

In general, the number of samples that should be collected within each project segment 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 the following factors should be considered:

- the greater the number of samples collected, the better the areal and/or vertical definition
- single measurements are inadequate to describe variability
- the means of several measurements at each station within a project segment generally are less variable than individual measurements at each station.

Time and Funding Constraints:

In all cases, the ultimate objective is to obtain sufficient information to evaluate the environmental impact of a dredged material disposal operation. The realities of time and funding constraints have to be recognized, although such do not justify inadequate environmental evaluation. Possible responses to cost 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, all of which increase the risk of an incorrect determination:

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

Project Segments:

Regardless of the final decision on project segments and the number of sample stations and replicates per project segment, expected or known degree of contamination will be the dominant factor in initially describing the proposed project segments. If variation in potential dredged material impact within a project segment is likely, where possible it may be advisable either to use a stratified random-sampling approach or to redefine project-segment boundaries. Once sampling data are available, it is advisable to reconsider the boundaries of the project segments to be used in the actual dredging in order to maximize homogeneity within segments.

Sample Compositing:

The objective of obtaining an accurate representation and definition of the dredging area and method has to be satisfied when compositing samples. Compositing provides a way to control cost while still analyzing sediments from a large number of stations. Compositing results in a less detailed description of variability within the area sampled than would individual analysis at each station. However 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 also provide the large sample volumes required for some biological tests. Composite samples represent the "average" of the characteristics of the individual samples making up the composite and are generally appropriate for logistic and other reasons; however, composite samples which serve to "dilute" a highly toxic but localized sediment "hot spot" are not recommended. Further, composite samples are not recommended for stations with very different sediment grain size characteristics.

Sample Definition:

When a sediment sample is collected, 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. For instance, based on observed stratification, the top 1 m 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 should be thoroughly homogenized. Samples may be split before compositing, with a portion of the original sediment archived for possible later analysis, and the remainder combined with parts of other samples. These are then thoroughly homogenized (using clean instruments until color and textural homogeneity are achieved), producing the composite sample.

8.2.5 Sample Collection Methods

Sample collection requires an adequately trained crew, an adequate vessel equipped with navigational and supporting equipment appropriate to the site and the study, and noncontaminating sampling apparatus capable of obtaining representative samples. Divers may also be used in some cases to collect some samples; in such cases divers must be certified and approved diver safety management plans must be in place. To assure sampling quality, at least one individual familiar with the study area should be present during the sampling activities. Sampling effort for a proposed dredging operation is primarily oriented toward collection of sediment samples for physical and chemical characterization and for biological tests. Collection of water samples is also 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 or to assess concentrations of contaminants in tissues. Organisms for use in toxicity and bioaccumulation tests may also be field-collected.

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. Where possible, the known, or expected, least contaminated stations should be sampled first. At a station where water and sediment are to be collected, water samples should be collected prior to sediment samples. The vessel should ideally be positioned downwind or downcurrent of the sampling device. When raising or lowering sampling devices, care should be taken to avoid visible surface slicks and the vessel's exhaust. The deck and sample handling area should be kept clean to help reduce the possibility of contamination.

8.2.5.1 Sediment Sample Collection

Mudroch and MacKnight (1991) provide useful reference information. Higgins and Lee (1987) provide a perspective on sediment collection and analysis as commonly practiced in USACE Districts. ASTM (1994a) and Burton (1991) provide guidelines for collecting sediments for toxicological testing. Guidance provided in these publications may be followed on all points that do not conflict with this manual.

Care should be taken to avoid contamination of sediment samples during collection and handling. A detailed procedure for handling sampling equipment and sample containers should be clearly stated in the sampling plan associated with a specific project. This may be accomplished by using standard operating procedures (SOPs). For example, samples designated for trace metal analysis should not come into contact with metal surfaces (except stainless steel, unless specifically prohibited for a project), 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 with appropriate liners is recommended whenever sampling to depth is required. The choice of corer design depends upon factors including the objectives of the sampling program, sediment volumes required for testing, sediment type, water depth, sediment depth, and currents or tides. 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 is generally preferable. These types of coring devices are generally limited to soft, unconsolidated sediments. A split-spoon core may be used for more compacted sediment. The length of core that can be collected is usually 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 or vibra corer should be used. Piston corers use 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. A vibra corer is a more complex piece of equipment but is capable of obtaining 3- to 7-m cores in a wide range of sediment types by vibrating a large diameter core barrel through the sediment column with little compaction. If the samples will not be sectioned prior to analysis, compaction is not a problem, and noncontaminating freefall 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 less likely in shallow channel areas without major direct contaminant inputs, that have frequent ship traffic, and from which sediments are dredged at short intervals. Generally, in these situations, accumulating sediments are resuspended and mixed semicontinuously by ship scour and turbulence, effectively preventing stratification. In such cases, surface 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 also appropriate for collecting surficial samples of reference or control sediments. A grab can be Teflon-coated to prevent potential contamination of trace metal samples. The sampling device should at least be rinsed with clean water between samples and possibly also solvent-rinsed.

8.2.5.2 Water Sample Collection

If water samples are necessary, representative samples should be collected with either a noncontaminating pump or a discrete water sampler. 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 epoxy-coated). Potential sample contamination must be avoided, including vessel emissions and other sampling apparatus.

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 (1:1 nitric acid) prior to use for collection of trace-metal samples, and solvent-rinsed prior to collection of samples for organic analyses.

8.2.5.3 Organism Collection

Benthic organism collection methods may be species specific and can include, but are not restricted to, bottom trawling, grabs or cores. If organisms are to be maintained alive, they should be transferred immediately to containers with clean, well-oxygenated water, and sediment as appropriate. Care must be taken to prevent organisms from coming into contact with potentially contaminated areas or fuels, oils, natural rubber, trace metals, or other contaminants.

8.2.6 Sample Handling, Preservation, and Storage

Detailed procedures for sample handling, preservation, and storage should be part of the standard operating procedures and protocols developed for each sampling operation. Samples are subject to chemical, biological, and physical changes as soon as they are collected. 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. Collection methods, volume requirements, container specifications, preservation techniques, storage conditions and holding times (from the time of sample collection) for sediment, water, and tissue samples are discussed below and summarized in Table 8-2.

8.2.6.1 Sample Handling

Sufficient sample volume must be collected to:

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

Analyses	Collection Method[b]	Amount Required[c]	Container[d]	Preservation Technique	Storage Conditions	Holding times[e]
SEDIMENT						
Chemical/Physical Analyses						
Metals	Grab/corer	100 g	Precleaned polyethylene jar[f]	Dry ice[f] or freezer storage for extended storages; otherwise refrigerate	≤ 4°C	Hg - 28 days Others - 6 months[g]
Organic compounds (e.g., PCBs, pesticides, polycyclic aromatic hydrocarbons)	Grab/corer	250 g	Solvent-rinsed glass jar with Teflon lid[f]	Dry ice[f] or freezer storage for extended storages; otherwise refrigerate	≤ 4°C[f]/dark[g]	14 days[h]
Particle size	Grab/corer	100 g	Whirl-pac bag[f]	Refrigerate	< 4°C	Undetermined
Total Organic Carbon (TOC)	Grab/corer	50 g	Heat treated glass vial with Teflon-lined lid[f]	Dry ice[f] or freezer storage for extended storages; otherwise refrigerate	≤ 4°C[f]	14 days
Total solids/ specific gravity	Grab/corer	50 g	Whirl-pac bag	Refrigerate	< 4°C	Undetermined
Miscellaneous	Grab/corer	≥ 50g	Whirl-pac bag	Refrigerate	< 4°C	Undetermined

Table 8-2 *(continued)*

Analyses	Collection Method[b]	Amount Required[c]	Container[d]	Preservation Technique	Storage Conditions	Holding times[e]
SEDIMENT (continued)						
Sediment from which elutriate is prepared	Grab/corer	Depends on tests being performed	Glass with Teflon-lined lid	Completely fill and refrigerate	4°C/dark/airtight	14 days
Biological Tests						
Dredged material	Grab/corer	12-15 L per sample	Plastic bag or container[i]	Completely fill and refrigerate; sieve	4°C/dark/airtight	14 days[j]
Reference sediment	Grab/corer	45-50 L per test	Plastic bag or container[i]	Completely fill and refrigerate; sieve	4°C/dark/airtight	14 days[j]
Control Sediment	Grab/corer	21-25 L per test	Plastic bag or container[i]	Completely fill and refrigerate; sieve	4°C/dark/airtight	14 days[j]
WATER AND ELUTRIATE						
Chemical/Physical Analyses						
Particulate analysis	Discrete sampler or pump	500 - 2000 mL	Plastic or glass	Lugols solution and refrigerate	4°C	Undetermined
Metals	Discrete sampler or pump	1 L	Acid-rinsed polyethylene or glass jar[k]	pH <2 with HNO_3[k]; refrigerate	4°C 2°C[k]	Hg - 14 days Others - 6 months[l]
Total Kjeldahl nitrogen (TKN)	Discrete sampler or pump	100 - 200 mL	Plastic or glass[l]	H_2SO_4 to pH <2; refrigerate	4°C[l]	24 h[l]
Chemical oxygen demand (COD)	Discrete sampler or pump	200 mL	Plastic or glass[l]	H_2SO_4 to pH <2; refrigerate	4°C[l]	7 days[l]

Table 8-2 *(continued)*

Analyses	Collection Method[b]	Amount Required[c]	Container[d]	Preservation Technique	Storage Conditions	Holding times[e]
WATER AND ELUTRIATE (continued)						
Total organic carbon (TOC)	Discrete sampler or pump	100 mL	Plastic or glass[l]	H_2SO_4 to pH <2; refrigerate	4°C[l]	<48 h[l]
Total inorganic carbon (TIC)	Discrete sampler or pump	100 mL	Plastic or glass[l]	Airtight seal; refrigerate[h]	4°C[l]	6 months[l]
Phenolic compounds	Discrete sampler or pump	1 L	Glass[l]	0.1 - 1.0 g $CuSO_4$; H_2SO_4 to pH <2; refrigerate	4°C[l]	24 h[l]
Soluble reactive phosphates	Discrete sampler or pump	-	Plastic or glass[l]	Filter; refrigerate[h]	4°C[l]	24 h[l]
Extractable organic compounds (e.g., semivolatiles)	Discrete sampler or pump	4 L	Amber glass bottle[k]	pH < 2, 6N HCl; airtight seal; refrigerate	4°C[k]	7 days for extraction; 40 days for extract analysis[k]
Volatile organic compounds	Discrete sampler or pump	80 mL	Glass vial[k]	pH < 2 with 1:1 HCL; refrigerate in airtight, completely filled container[k]	4°C[k]	14 days for sample analysis if preserved[m]
Total phosphorus	Discrete sampler or pump	-	Plastic or glass[i]	H_2SO_4 to pH < 2; refrigerate	4°C[l]	7 days[l]
Total solids	Discrete sampler or pump	200 mL	Plastic or glass[l]	Refrigerate	4°C[l]	7 days[l]

Table 8-2 *(continued)*

Analyses	Collection Method[b]	Amount Required[c]	Container[d]	Preservation Technique	Storage Conditions	Holding times[e]
WATER AND ELUTRIATE (continued)						
Volatile solids	Discrete sampler or pump	200 mL	Plastic or glass[l]	Refrigerate	4°C[l]	7 days[l]
Sulfides	Discrete sampler or pump	-	Plastic or glass[l]	pH > 9 NaOH (ZnAc); refrigerate	4°C[l]	24 h[l]
Biological Tests						
Site water	Grab	Depends on tests being performed	Plastic carboy	Refrigerate	< 4°C	14 days
Dilution water	Grab or makeup	Depends on tests being performed	Plastic carboy	Refrigerate	<4°C	14 days
TISSUE						
Metals	Trawl/Teflon-coated grab	5-10 g	Double Ziploc[f]	Handle with nonmetallic forceps; plastic gloves; dry ice[f]	≤ -20°C[f] or freezer storage	Hg - 28 days Others - 6 months[n]
PCBs and chlorinated pesticides	Trawl/Teflon-coated grab	10-25 g	Hexane-rinsed double aluminum foil and double Ziploc[f]	Handle with hexane-rinsed stainless steel forceps; dry ice[f]	≤ -20°C[f] or freezer storage	14 days[h]
Volatile organic compounds	Trawl/Teflon-coated grab	10-25 g	Heat-cleaned aluminum foil and watertight plastic bag[m]	Covered ice chest[g]	≤ -20°C[h] or freezer storage	14 days[n]

Table 8-2 *(continued)*

Analyses	Collection Method[b]	Amount Required[c]	Container[d]	Preservation Technique	Storage Conditions	Holding times[e]
TISSUE (continued)						
Semivolatile organic compounds (e.g, PAH)	Trawl/Teflon-coated grab	10-25 g	Hexane-rinsed double aluminum foil and double Ziploc[f]	Handle with hexane-rinsed stainless steel forceps; dry ice[f]	≤ -20°C[f] or freezer storage	14 days[h]
Lipids	Trawl/Teflon-coated grab	part of organic analyses	Hexane-rinsed aluminum foil	Handle with hexane-rinsed stainless steel forceps; quick freeze	≤ -20°C or freezer storage	14 days[h]

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 Collection method should include appropriate liners.

c Amount of sample required by the laboratory to perform the analysis (wet weight or volume provided, as appropriate). Miscellaneous sample size for sediment should be increased if auxiliary analytes that cannot be included as part of the organic or metal analyses are added to the list. The amounts shown are not intended as firm values; more or less tissue may be required depending on the analytes, matrices, detection limits and particular analytical laboratory.

d All containers should be certified as clean according to EPA (1990a).

e These holding times are for sediment, water, and tissue based on guidance that is sometimes administrative rather than technical in nature. There are no promulgated, scientifically based holding time criteria for sediments, tissues or elutriates. References should be consulted if holding times for sample extracts are desired. Holding times are from the time of sample collection.

f NOAA (1989)

g Tetra Tech (1986a)

h Sample may be held for up to one year if ≤ -20°C.

i Polypropylene should be used if phthalate bioaccumulation is of concern.

j Two weeks is recommended; sediments must not be held for longer than 8 weeks prior to biological testing.

k EPA (1987c); 40 CFR Part 136, Table III

l Plumb (1981)

m If samples are not preserved to pH<2, then aromatic compounds must be analyzed within 7 days.

n Tetra Tech (1986b)

- perform the necessary analyses
- partition the samples, either in the field or as soon as possible after sampling, for respective storage and/or analytical requirements (e.g., freezing for trace metal analysis, refrigeration for bioassays)
- provide sample for replicate or QA analyses, if specified
- archive portions of the sample for possible later analysis.

Sample handling is project and analysis specific as well as being based on what is practical and possible. 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).

For analysis of volatile compounds, samples should completely fill the storage container, leaving no air-space. These samples should be refrigerated but never frozen or the containers will crack. Samples for other kinds of chemical analysis are sometimes frozen. If the sample is to be frozen, sufficient 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 labelling system should be tested prior to use in the field.

Sediment samples for biological testing should have at least the larger living organisms removed from the sediment prior to testing. This may be 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, individual test sediments should be thoroughly homogenized with clean instruments (until color and textural homogeneity is achieved).

8.2.6.2 Sample Preservation

Preservation steps should be taken immediately upon sediment collection. There is no universal preservation or storage technique although storage in the dark at 4°C is generally used for all samples held for any length of time prior to partitioning, and for some samples after partitioning. 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 its integrity.

Onboard refrigeration is generally accomplished with coolers and ice; however, samples should be segregated from melting ice or cooling water. Samples which are to be frozen on board may be stored in an onboard freezer or may simply be placed in a cooler with dry ice or blue ice. Sediment samples for biological analysis should be preserved at 4°C, never frozen or dried. Additional guidance on sample preservation is given in Table 8-2.

8.2.6.3 Sample Storage

The elapsed time between sample collection and analysis should be as short as possible. Sample holding times for chemical evaluations are analysis-specific (Table 8-2). Sediments for bioassay (toxicity and/or bioaccumulation) testing *should* be tested as soon as possible, preferably within 2 weeks of collection. Studies to date suggest that sediment storage time should not exceed 8 weeks (at 4°C, in the dark, excluding air) (Becker and Ginn, 1990; Tatem et al., 1991). Toxicity may change with storage time. Sample storage conditions (e.g., temperature, location of samples) should be documented.

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 (e.g., see EPA, 1995). Contingency plans are essential. Well-trained, qualified, 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 for more efficient work than an overcrowded vessel. Unforeseeable circumstances (e.g., weather delays) are to be expected during field sampling, and time to adequately accomodate the unforeseen has to be included in sampling schedules.

Appropriate safety and health precautions must be observed during field sampling activities. EPA (1984) should be used as a guidance document to prepare a site-specific health and safety plan. The health and safety plan should be prepared as a separate document from the QA project plan. Requirements set forth in the Occupational Safety and Health Administration 29 CFR § 1910.120 (Federal Register, Vol. 54, No. 43) should be met for medical surveillance, personal protection, respirator fit testing (if applicable),

and hazardous waste operations training (if applicable) by all personnel working in contaminated areas or working with contaminated media.

The procedures and practices established in the site-specific health and safety plan must be observed by all individuals participating in the field activities. Safety requirements should also be met by all observers present during field audits and inspections. The plan should include the following information:

- site location and history
- scope of work
- site control
- hazard assessment (chemical and physical hazards)
- levels of protection and required safety equipment
- field monitoring requirements
- decontamination
- training and medical monitoring requirements
- emergency planning and emergency contacts.

Samples must be properly disposed when no longer needed. Ordinary sample-disposal methods are usually acceptable, and special precautions are seldom appropriate. Under Federal law [40 CFR 261.5(a)], where highly contaminated wastes are involved, 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 1,000 Kg of waste on the property without being subject to the requirements of Federal hazardous waste regulations. However, State and local regulations may require special handling and disposal of contaminated samples. 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 Non-Indigenous Test Species

Over the last few years, there has been a growing awareness of the ecological and economic damage caused by introduced species. Because both east and west coast species are often used in bioaccumulation

tests, there is a real potential of introducing bioaccumulation test species or associated fauna and flora (e.g., pathogens, algae used in transporting the worms). It is the responsibility of the persons conducting the bioaccumulation or toxicity tests to assure that no non-indigenous species are released.

The general procedures to contain non-indigenous species are to collect and then poison all water, sediment, organisms and associated packing materials (e.g., algae, sediment) before disposal. Chlorine bleach can be used as the poison. A double containment system is used to keep any spillage from going down the drain. Guidance on procedures used in toxicity tests can be found in Appendix B of DeWitt et al. (1992a). Flow-through tests can generate large quantities of water, and researchers should plan on having sufficient storage facilities.

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

This section provides guidance on the selection of chemical and physical analyses to aid in the evaluation of dredged material for proposed disposal, and on the methods used to analyze these parameters. QA/QC guidance is provided in Appendix G and EPA (1995).

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, proven, 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 analysis of sediment and water from estuarine or marine environments must explicitly address steps taken to control salt interference.*

9.1 Physical Analysis of Sediment

Physical characteristics of the dredged material must be determined to help assess the impact of disposal on the benthic environment and the water column at the disposal site. This is the first step in the overall process of sediment characterization, and also helps to identify appropriate control and reference sediments for biological tests. In addition, physical analyses can be helpful in evaluating the results of analyses and tests conducted later in the characterization process.

The general analyses may include (1) grain size, (2) total solids and (3) specific gravity.

Grain-size analysis defines the frequency distribution of the size ranges of the particles that make up the project sediment (e.g., 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. Use of the Unified Soil Classification System (USCS) for physical characterization is recommended for the purpose of consistency with USACE engineering evaluations (ASTM, 1992).

Total solids is a gravimetric determination of the organic and inorganic material remaining in a sample after it has been dried at a specified temperature. The total solids values generally are used to convert concentrations of contaminants 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). The specific gravity of a dredged-material sample helps to predict the behavior (i.e., dispersal and settling characteristics) of dredged material after disposal.

Other physical/engineering properties (e.g., Atterburg limits, hydrometer analysis, settling properties, etc.) may be needed to evaluate the quality of any effluent discharged from confined disposal facilities. Guidance in this regard is provided in Appendix B.

9.2 Target Detection Limits

The selection of appropriate target detection limits (TDLs) is vital (e.g., TetraTech, 1986a; EPA, 1986a). TDLs should be lower than the appropriate values against which the data are to be compared for interpretation. Different analytical methods are capable of detecting different concentrations of a chemical in a sample. For example, a highly sensitive technique can detect a much lower chemical concentration than can a screening technique for the same chemical. The accuracy of measurements also differs among analytical techniques. In general, as the sensitivity and accuracy of a technique increases, so does the cost. Recommended TDLs that are judged to be feasible, cost effective, and to meet the requirements for dredged material evaluations are summarized in EPA (1995), along with example analytical methods that are capable of meeting those TDLs. However, any method that can achieve those TDLs is acceptable, provided that the appropriate documentation of the method performance is generated for the project.

The TDL is a performance goal set between the lowest, technically feasible detection limit for routine analytical methods and available regulatory criteria or guidelines for evaluating dredged material. The TDL is, therefore, equal to or greater than the lowest amount of a chemical that can be reliably detected based on the variability of the blank response of routine analytical methods (see EPA [1995] for discussion of method blank response). However, the reliability of a chemical measurement generally increases as the concentration increases. Analytical costs may also be lower at higher detection limits. For these reasons, the TDLs in EPA (1995) have been set at not less than 10 times lower than available regional or international dredged material guidelines for potential biological effects associated with sediment chemical contamination.

All data generated for dredged material evaluation should meet the TDLs in EPA (1995) unless prevented by sample-specific interferences. Any sample-specific interferences must be well documented by the laboratory. If significantly higher or lower TDLs are required to meet rigorously defined data quality

objectives (e.g., for human health risk assessments) for a specific project then, on a project-specific basis, modification to existing analytical procedures may be necessary. Such modifications must be documented in the QA project plan. An experienced analytical chemist should be consulted so the most appropriate method modifications can be assessed, the appropriate coordination with the analytical laboratory can be implemented, and the data quality objectives can be met. A more detailed discussion of method modifications is provided in EPA (1995).

9.3 Chemical Analysis of Sediment

9.3.1 Target Analytes

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) establishes a reason to believe that sediment contaminants may be present, but fails to produce sufficient information to develop a definitive list of potential contaminants, a list of target analytes has to be compiled. Target analytes should be selected from, but not necessarily limited to, the compounds in Table 9-1 and from the historical review information. The target list should include contaminants that historical information or commercial and/or agricultural applications suggest could be present at a specific dredging site — for example, tributyltin near shipyards, berthing areas, and marinas where these compounds have been applied. 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).

All PCB analyses should be made using congener-specific methods. The sum of the concentrations of specific congeners is an appropriate measure of total PCBs (NOAA, 1989).

Sediments should be analyzed for total organic carbon (TOC). This is particularly important if there are hydrophobic organics on the contaminant of concern list developed in Tier I. The TOC content of sediment is a measure of the total amount of oxidizable organic material in a sample and also affects contaminant bioaccumulation by, and effects to, organisms (e.g., Di Toro et al., 1991; DeWitt et al., 1992b).

Table 9-1. Potential Contaminants of Concern Listed According to Structural Compound Class.

Structural Compound Class	Contaminant
Phenols	phenol
	2,4-dimethylphenol
	2-methylphenol
	4-methylphenol
Substituted Phenols	2,4,6-trichlorophenol
	para-chloro-meta-cresol
	2-chlorophenol
	2,4-dichlorophenol
	2-nitrophenol
	4-nitrophenol
	2,4-dinitrophenol
	4,6-dinitro-*o*-cresol
	pentachlorophenol
Organonitrogen Compounds	benzidine
	3,3'-dichlorobenzidine
	2,4-dinitrotoluene
	2,6-dinitrotoluene
	1,2-diphenylhydrazine
	nitrobenzene
	N-nitrosodimethylamine
	N-nitrosodiphenylamine
	N-nitrosodipropylamine
Low Molecular Weight Polynuclear Aromatic Hydrocarbons (PAH)	acenaphthene
	naphthalene
	acenaphthylene
	anthracene
	phenanthrene
	fluorene
	1-methylnapthalene
	2-methylnapthalene
High Molecular Weight Polynuclear Aromatic Hydrocarbons (PAH)	fluoranthene
	benzo*(a)*anthracene
	benzo*(a)*pyrene
	benzo*(b)*fluoranthene
	benzo*(k)*fluoranthene
	chrysene
	benzo*(ghi)*perylene
	dibenzo*(a,h)*anthracene
	ideno(1,2,3-*cd*)pyrene
	pyrene
Chlorinated Aromatic Hydrocarbons	1,2,4-trichlorobenzene
	hexachlorobenzene
	2-chloronaphthalene
	1,2-dichlorobenzene
	1,3-dichlorobenzene
	1,4-dichlorobenzene
Chlorinate Aliphatic Hydrocarbons	hexachlorobutadiene
	hexachloroethane
	hexachlorocyclopentadiene
Halogenated Ethers	bis(2-chloroethyl)ether
	4-chlorophenyl ether
	4-bromophenyl ether
	bis(2-chloroisopropyl) ether
	bis(2-chlorethoxy)methane
Phthalates	bis(2-ethylhexyl)phthalate
	butyl benzyl phthalate
	di-*n*-butyl phthalate
	di-*n*-octyl phthalate
	diethyl phthalate
	dimethyl phthalate
Polychlorinated Biphenyls (PCB) as Aroclors[a]	PCB-1242
	PCB-1254
	PCB-1221
	PCB-1232
	PCB-1248
	PCB-1260
	PCB-1016
Miscellaneous Oxygenated Compounds	TCDD (dioxin)[b]
	PCDF (furan)
	isophorone
Pesticides	aldrin
	dieldrin
	chlordane
	chlorbenside
	dacthal
	DDT[c]
	endosulfan[d]
	endrin
	endrin aldehyde
	heptachlor
	heptachlor epoxide
	α-hexachlorocyclohexane
	β-hexachlorocyclohexane
	δ-hexachlorocyclohexane
	γ-hexachlorocyclohexane
	toxaphene
	mirex
	methoxychlor
	parathion
	malathion
	guthion
	demeton

Table 9-1. *(continued)*

Structural Compound Class	Contaminant
Volatile Halogenated Alkanes	tetrachloromethane
	1,2-dichloroethane
	1,1,1-trichloroethane
	1,1-dichloroethane
	1,1,2-trichloroethane
	1,1,2,2-tetrachloroethane
	chloroethane
	chloroform
	1,2-dichloropropane
	dichloromethane
	chloromethane
	bromomethane
	bromoform
	dichlorobromoethane
	fluorotrichloromethane
	dichlorodifluoromethane
	chlorodibromomethane
Volatile Halogenated Alkenes	1,1-dichlorethylene
	1,2-*trans*-dichlorethylene
	trans-1,3-dichloropropene
	cis-1,3-dichloropropene
	tetrachlorethene
	trichlorethene
	vinyl chloride
Volatile Aromatic Hydrocarbons	benzene
	ethylbenzene
	toluene
Chlorinated Benzenes	1,3-dichlorobenzene
	1,4-dichlorobenzene
	1,2-dichlorobenzene
	1,2,4-trichlorobenzene
	hexachlorobenzene
Volatile Unsaturated Carbonyl Compounds	acrolein
	acrylonitrile
Volatile Ethers	2-chlorethylvinylether
	bis(chloromethyl)ether
Metals	aluminum
	antimony
	arsenic
	beryllium
	butyltins
	cadmium
	chromium (hexavalent)
	cobalt
	copper
	iron
	lead
	manganese
	mercury
	nickel
	selenium
	silver
	thallium
	tin
	zinc
Miscellaneous	ammonia[e]
	asbestos
	benzoic acid
	cyanide
	guaiacols
	methylethyl ketone
	resin acids

[a]It is recommended that PCB analyses use congener-specific methods. The sum of the concentrations of specific congeners is an appropriate measure of total PCBs (see Table 9-3).
[b]Additional dioxin and furan (e.g., TCDF) compounds are listed in Table 9-2.
[c]Includes DDT, DDD, and DDE
[d]Includes ∝-endosulfan, β-endosulfan, and endosulfan sulfate.
[e]Ammonia may not be a contaminant of concern at certain open-water dredged material disposal sites (e.g., dispersive situations and situations with well-oxygenated overlying water).

Sediments in which metals are suspected to be contaminants of concern may also be analyzed for acid volatile sulfide (AVS) (Di Toro et al., 1990; EPA, 1991a). Although acceptable guidance on the interpretation of AVS measurements is not yet available, and AVS measurements are not generally recommended at this time, such measurements can provide information on the bioavailability of metals in anoxic sediments. Presently, AVS studies represent an area of on-going research which may be formally included in the manual if and when decision criteria are determined.

9.3.2 Selection of Analytical Techniques

Once the list of target analytes for sediments has been established, analytical methods have to be determined. The methods will, to some degree, dictate the amount of sediment sample required for each analysis. General sample sizes are provided in Table 8-2, and include possible requirements for more than one analysis for each group of analytes. The amount of sample used in an analysis affects the detection limits attainable by a particular method.

TOC analyses 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. Acceptable methods for TOC analysis are available from EPA (1995).

For many metals analyses in marine/estuarine areas, the concentration of salt may be much greater than the analyte of interest and can cause unacceptable interferences in certain analytical techniques. In such cases, the freshwater approach of acid digestion followed by inductively coupled plasma-atomic emission spectrometry (ICP) or graphite furnace atomic absorption spectroscopy (GFAAS) should be coupled with appropriate techniques for controlling this interference. The Hg method in EPA (1986a; Method 7471) may be used for the analysis of Hg in sediment. Tributyltin may be analyzed by the method of Rice et al. (1987), and selenium and arsenic by the method of EPRI (1986). A total extraction of metal ions is neither necessary nor desirable for dredged material evaluations. The standard aqua regia extraction yields consistent and reproducible results.

The recommended method for 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): gas chromatography/mass spectrometry (GC/MS) techniques for semi-volatile and volatile priority pollutants, and dual column gas chromatography/electron-capture detection (GC/ECD) for pesticides and PCBs (NOAA, 1989). Alternatively, GC/MS using selected ion monitoring can be used for PCB and pesticide analysis. These analytically sound techniques yield accurate data on the concentrations of chemicals in the sediment matrix. The analytical techniques for semivolatile organic compounds generally involve solvent extraction from the sediment matrix and subsequent analysis, after cleanup, using GC or GC/MS. 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 (i.e., 2,3,7,8, - TCDD) analysis is being performed, the methods of Kuehl et al. (1987), Smith et al. (1984), EPA (1989b; Method 8290), or EPA (1990c; Method 1613) and summary in EPA (1995) should be consulted. EPA Method 1613 is the recommended procedure for measuring the tetra- through octa- chlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). This method has been developed for analysis of water, soil, sediment, sludge, and tissue. Table 9-2 shows the 17 compounds determined by Method 1613.

Techniques for analysis of chemical constituents have some inherent limitations for sediment samples. Interferences encountered as part of the sediment matrix, particularly in samples from heavily contaminated areas, may limit the ability of a method to detect or quantify some analytes. The most selective methods using GC/MS techniques are recommended for all nonchlorinated organic compounds because such 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 PCB and pesticide analyses because GC/ECD analysis will result in lower detection limits. The analysis and identification of PCBs by GC/ECD methods are based upon relative retention times and peak shapes. Matrix interferences may result in the reporting of false negatives, although congener-specific PCB analysis reduces this concern relative to use of the historical Aroclor® matching procedure.

PCBs have traditionally been quantified with respect to Aroclor® mixtures. This procedure can result in errors in determining concentrations (Brown et al., 1984). For dredged material evaluations, the concentration of total PCBs should be determined by summing the concentrations of specific individual PCB congeners identified in the sample (see Table 9-3). The minimum number of PCB congeners that should be analyzed are listed in the first column of Table 9-3 (i.e., "summation" column) (NOAA, 1989). This summation is considered the most accurate representation of the PCB concentration in samples. Additional PCB congeners are also listed in Table 9-3. McFarland and Clarke (1989) recommend these PCB congeners for analysis based on environmental abundance, persistence, and biological importance.

Table 9-2. PCDD and PCDF Compounds Determined by Method 1613

Native Compound[1]
2,3,7,8-TCDF
2,3,7,8-TCDD
1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF
1,2,3,7,8-PeCDD
1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF
1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD
1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDF
1,2,3,4,6,7,8-HpCDD
1,2,3,4,7,8,9-HpCDF
OCDD
OCDF

[1] Polychlorinated dioxins and furans:

TCDD = Tetrachlorodibenzo-p-dioxin
TCDF = Tetrachlorodibenzofuran
PeCDD = Pentachlorodibenzo-p-dioxin
PeCDF = Pentachlorodibenzofuran
HxCDD = Hexachlorodibenzo-p-dioxin
HxCDF = Hexachlorodibenzofuran
HpCDD = Heptachlorodibenzo-p-dioxin
HpCDF = Heptachlorodibenzofuran
OCDD = Octachlorodibenzo-p-dioxin
OCDF = Octachlorodibenzofuran

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

PCB Congener[a]	Congener Number[b]		
	Summation[c]	Highest Priority[d]	Second Priority[e]
2,4' diCB	8		
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[f]	126[f]	
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[f]	169[f]	
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

(continued)

Table 9-3. *(continued)*

PCB Congener[a]	Congener Number[b]		
	Summation[c]	Highest Priority[d]	Second Priority[e]
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 Mullin 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).

[f]To separate PCBs 126 and 169, it is necessary to initially utilize an enrichment step with an activated carbon column (Smith, 1981).

McFarland et al. (1986) note that the most toxic PCB congeners lie mainly within the tetra-, penta-, and hexa- chlorobiphenyl groups. Sample preparation for PCB congener analysis should follow the techniques described by Tetra Tech (1986a) or EPA (1986a), but with instrumental analysis and quantification using standard capillary GC columns on individual PCB isomers according to the methods reported by NOAA (1989) (see also Dunn et al., 1984; Schwartz et al., 1984; Mullin et al., 1984; Stalling et al., 1987).

Although the methods mentioned above are adequate for detecting and quantifying concentrations of those PCB congeners comprising the majority of total PCBs in environmental samples, they are not appropriate for separating and quantifying PCB congeners which may coelute with other congeners and/or may be present at relatively small concentrations in the total PCB mixture. Included in this latter group of compounds, for example, are PCBs 126 and 169, two of the more toxic nonortho-substituted (coplanar) PCB congeners (Table 9-3). In order to separate these (and other toxic nonortho-substituted congeners), it is necessary to initially utilize an enrichment step with an activated carbon column (Smith, 1981). Various types of carbon columns have been used, ranging from simple gravity columns (e.g., in a Pasteur pipette) to more elaborate (and efficient) columns using high pressure liquid chromatography (HPLC) systems (see Schwartz et al., 1993). The preferred method of separation and quantitation of the enriched PCB mixture has been via high resolution GC-MS with isotope dilution (Kuehl et al., 1991; Ankley et al., 1993; Schwartz et al., 1993). However, recent studies have shown that if the carbon enrichment is done via HPLC, the nonortho-substituted PCB congeners of concern also may be quantifiable via more widely available GC/ECD systems (Schwartz et al., 1993).

The overall toxicity of nonortho-substituted PCBs at a site can be assessed based on a comparison with the toxicity of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). A similar procedure can be used for assessing the toxicity of a mixture of dioxins and furans. In this "toxicity equivalency factor" (TEF) approach, potency values of individual congeners (relative to TCDD) and their respective sediment concentrations are used to derive a "summed" 2,3,7,8-TCDD equivalent (TCDD-EQ) (EPA, 1989c; Table 9-4). Ankley et al. (1992b) provide an example of the use of this approach.

TEFs have been derived for human health purposes. For aquatic organisms the relative toxicities of different PCB congeners and dioxins are likely to be quite different. For instance, wildlife or fish TEF for PCBs are not equivalent to those for humans (Walker et al., 1992).

To ensure that contaminants not included in the list of target analytes are not overlooked in the chemical characterization of the dredged material, the analytical results should also be scrutinized by trained personnel. The presence of persistent major unknown analytes should be noted. Methods involving GC/MS techniques for organic compounds are recommended for the identification of any unknown analytes.

Table 9-4. Methodology for Toxicity Equivalency Factors

Because toxicity information on some dioxin and furan species is scarce, a structure-activity relationship has been assumed. The toxicity of each congener is expressed as a fraction of the toxicity of 2,3,7,8 TCDD.

Compound	TEF
2,3,7,8 TCDD	1
other TCDD	0
2,3,7,8-PeCDDs	0.5
other PeCDDs	0
2,3,7,8-HxCDDs	0.1
other HxCDDs	0
2,3,7,8-HpCDDs	0.01
other HpCDDs	0
OCDD	0.001
2,3,7,8-TCDF	0.1
other TCDFs	0
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
other PeCDFs	0
2,3,7,8-HxCDFs	0.1
other HxCDFs	0
2,3,7,8-HpCDFs	0.01
other HpCDFs	0
OCDF	0.001

9.4 Chemical Analysis of Water

9.4.1 Analytical Targets

Analysis to determine the potential release of dissolved contaminants from the dredged material (standard elutriate) may be necessary to make a factual determination. 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 and centrifugation. Chemical analysis of the elutriate allows a direct comparison, after allowance for mixing, to applicable water quality standards (WQS). When collecting samples for elutriate testing, consideration should be given to adequate volumes of water and sediment required to prepare samples for analysis including replicates where appropriate. In some instances, when there is poor settling, the elutriate preparation has to be performed successively several times to accumulate enough water for testing.

Historical water quality information from the dredging site (Tier I) should be evaluated along with data obtained from the chemical analysis of sediment samples to select target analytes. Chemical evaluation of the dredged material provides a known list of constituents which might affect the water column. All target analytes identified in the sediment should initially be considered potential targets for water analysis. Nonpriority-pollutant chemical components which are found in measurable concentrations in the sediments should be included as targets 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/or are of toxicological concern.

9.4.2 Analytical Techniques

In contrast to freshwater, there generally are no EPA approved methods for analysis of saline water although widely accepted methods have existed for some time (e.g., Strickland and Parsons, 1972; Grasshoff et al., 1983; Parsons et al., 1984). Application of the freshwater methods to saltwater will frequently result in higher detection limits than are common for freshwater unless care is taken to control the effects of salt on the analytical signal. 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 target detection limits (TDLs). It is extremely important to ascertain a laboratory's ability to execute methods and attain acceptable detection limits in matrices containing up to 3% sodium chloride.

Once the list of target analytes for water has been established, analytical methods have to be determined. The water volume required for specific analytical methods may vary. A minimum of 1 L of elutriate should be prepared for metals analysis (as little as 100 mL may be analyzed). One liter of elutriate should be analyzed for organic compounds. Sample size should also include the additional volume required for the matrix spike and matrix spike duplicate analyses required as part of the analytical procedure. Samples from the dredging site and, where appropriate, disposal site, should be delivered for organic and metals analysis. Sample size is one of the limiting factors in determining detection limits for water analyses, but TDLs below the WQS must be the goal in all cases. Participating laboratories should routinely report detection limits achieved for a given analyte.

Detailed methods for the analysis of organic and inorganic priority pollutants in water are referenced in 40 CFR 136 and in EPA (1983). Additional approved methods include EPA (1986a,b; 1988a,b,c; 1990b,c); APHA (1989); ASTM (1991b); Tetra Tech (1985). Most of these methods will require modification to achieve low detection limits in saline waters. 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. The volatile priority pollutants are determined by using purge-and-trap techniques and are analyzed by either GC or GC/MS. If dioxin (i.e., 2,3,7,8, - TCDD) analysis is necessary, Kuehl et al. (1987), Smith et al. (1984), EPA (1989b; Method 8290), or EPA (1990c; Method 1613) should be consulted. EPA Method 1613 is the recommended procedure for measuring the tetra- through octa-PCDDs and PCDFs.

A primary requirement for analysis of inorganic and organic priority pollutants is to obtain detection limits which will result in usable, quantitative data that can subsequently be compared against applicable WQS to determine compliance with the water quality certification requirement under Section 401. Existing EPA methods for freshwater analysis need to be adapted to achieve environmentally meaningful detection limits in saline waters because of matrix interferences caused by salt. 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 detection limits. 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. PCBs 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 (NOAA, 1989).

Analysis of saline water for metals is subject to matrix interferences from salts, particularly sodium and chloride ions, when the samples are concentrated prior to instrumental analysis. The gold-amalgamation method using cold-vapor atomic absorption spectrophotometry (AAS) analysis is recommended to eliminate saline water matrix interferences for mercury analysis. Methods using solvent extraction and

AAS analysis may be required to reduce saline water matrix interferences for other target metals. Other methods appropriate for metals include: cadmium, copper, lead, iron, zinc, silver (Danielson et al., 1978); arsenic (EPRI, 1986); selenium and antimony (Sturgeon et al., 1985); low levels of mercury (Bloom et al., 1983); and, tributyltin (Rice et al., 1987). Graphite-furnace AAS techniques after extraction are recommended for the analysis of metals, with the exception of mercury.

9.5 Chemical Analysis of Tissues

9.5.1 Target Analytes

Bioaccumulation is evaluated by analyzing tissues of test organisms for contaminants determined to be of concern for a specific dredged material. Sediment contaminant data and available information on the bioaccumulation potential of those analytes have to be interpreted to establish target compounds.

The *n*-octanol/water partition coefficient (K_{ow}) is used to estimate the BCFs of chemicals in organism/water systems (Chiou et al., 1977; Kenaga and Goring, 1980; Veith et al., 1980; Mackay, 1982). The potential for bioaccumulation generally increases as K_{ow} increases, particularly 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 considered for further evaluation of bioaccumulation potential. 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, but exceptions include the chlorinated benzenes and the chlorinated phenols. Table 9-5 provides data for organic priority pollutants based on K_{ow}. Specific target analytes for PCBs and PAHs are discussed in Section 9.3.1. The water content and percent lipids should be routinely determined as part of tissue analyses for organic contaminants.

Table 9-6 ranks the bioaccumulation potential of the inorganic priority pollutants based on calculated BCFs. Dredged material contaminants with BCFs greater than 1,000 (log BCF >3) should be further evaluated for bioaccumulation potential.

Tables 9-5 and 9-6 should 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

Table 9-5. Octanol/Water Partition Coefficients (K_{ow}) for Organic Compound Priority Pollutants and 301(h) Pesticides[a].

Pollutant	Octanol/Water Partition Coefficient (log K_{ow})	Pollutant	Octanol/Water Partition Coefficient (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

Table 9-5. *(continued)*

Pollutant	Octanol/Water Partition Coefficient (log K_{ow})	Pollutant	Octanol/Water Partition Coefficient (log K_{ow})
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
Chloroform	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.

[Note: Mixtures, such as PCB Aroclors®, cannot have discrete K_{ow} values, however, the value given is a rough estimate for the mean. It is recommended that all PCB analyses use congener-specific methods. All PCB congeners have a log K_{ow} >4 (L. Burkhardt, EPA Duluth, pers. comm.).]

Table 9-6. Bioconcentration Factors (BCF) of Inorganic Priority Pollutants.[a]

Inorganic 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.

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 for tissue analysis should include three considerations, all of which are related to regulatory concern:

- the target analyte is a contaminant of concern and is present in the sediment as determined by sediment chemical analyses
- the target analyte has a high potential to accumulate and persist in tissues
- the target analyte is of toxicological concern.

Contaminants with a lower potential to bioaccumulate, but which are present at high concentrations in the sediments, should also be included in the target list because bioavailability can increase with concentration. Conversely, contaminants with a high accumulation potential and of high toxicological concern should be considered as targets, even if they are only present at low concentrations in the sediment. Nonpriority-pollutant contaminants which are found in measurable concentrations in the sediments should be included as targets for tissue analysis if they have the potential to bioaccumulate and persist in tissues, and are of toxicological concern.

9.5.2 Analytical Techniques

At present, formally approved standard methods for the analysis of priority pollutants and other contaminants in tissues are not available. However, 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 dependent on the analytical procedure and the tissue moisture content. General guidance, but *not* firm recommendations, for the amount of tissue required, is provided in Table 8-2. The required amounts may vary depending on the analytes, matrices, detection limits, and particular analytical laboratory. Tissue moisture content must be determined for each sample to convert applicable data from a wet-weight to a dry-weight basis, however both wet- and dry-weight data should be reported.

Detection limits depend on the sample size as well as the specific analytical procedure. TDLs should be determined for all analytes according to initial guidance in 40 CFR 136 and more definitive guidance in

EPA (1995; cf. Section 9.2). Detection limits should be specified based on the intended use of the data and specific needs of each evaluation.

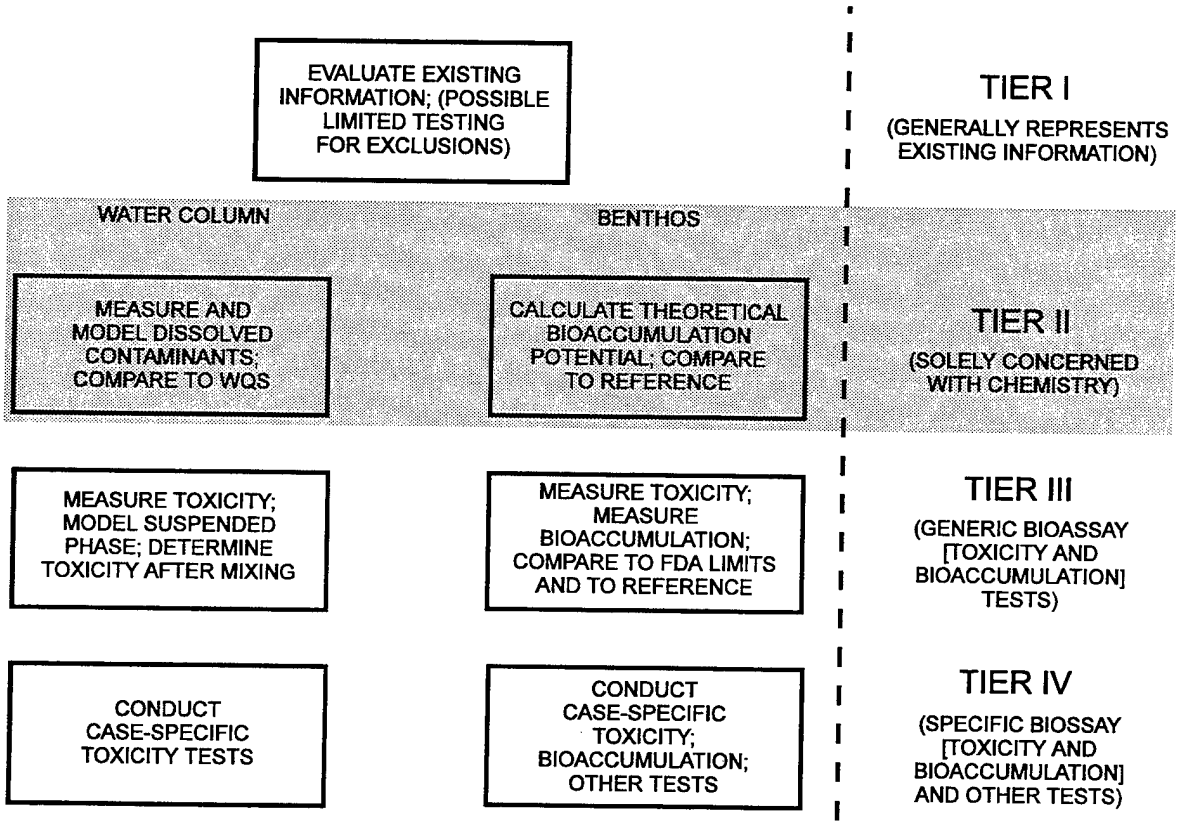
Existing methods for priority pollutant tissue analysis involve two separate procedures: one for organic compounds and another for metals. The recommended methods for the analysis of semivolatile organic pollutants are described in NOAA (1989). The procedure involves serial extraction of homogenized 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 Sloan et al. (1993) is recommended for rapid, efficient, reproducible sample cleanup. The extract is concentrated and analyzed for semivolatile organic pollutants using GC with capillary fused-silica columns to achieve sufficient analyte resolution. If dioxin (i.e., 2,3,7,8-TCDD) analysis is being performed, the methods of Mehrle et al. (1988), Kuehl et al. (1987), Smith et al. (1984), EPA (1989b; Method 8290), or EPA (1990c; Method 1613) should be consulted. EPA Method 1613 is the recommended procedure for measuring the tetra- through octa-PCDDs and PCDFs.

Chlorinated hydrocarbons (e.g., PCBs and chlorinated pesticides) should be analyzed by GC/ECD. PCBs should 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 PCBs in Section 9.3.2). Lower detection limits and positive identification of PCBs and pesticides can be obtained by using chemical ionization mass spectrometry.

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, 1986a). Lowest detection limits are achieved by operating the mass spectrometer in the SIM mode. Decisions to perform analysis of nonchlorinated hydrocarbons and resulting data interpretation should consider that many of these analytes are readily metabolized by most fish and many invertebrates. Analytical methods for analysis of tissue samples for volatile priority pollutants are found in Tetra Tech (1986b).

Tissue lipid content is of importance in the interpretation of bioaccumulation information. A lipid determination should be performed on biota submitted for organic analysis if: (1) food chain models will be used; (2) test organisms could spawn during the test; (3) special circumstances occur (Tier IV), such as those requiring risk assessment. Bligh and Dyer (1959) provide an acceptable method, and the various available methods are evaluated by Randall et al. (1991).

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-atomic emission spectrometry (ICP) techniques. Procedures in Tetra Tech (1986b) and EPA (1991c) are generally recommended. NOAA (1989) methods may also be used and are recommended when 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 by NOAA (1989), except for the microwave heating steps. Mercury analysis requires the use of cold-vapor AAS methods (EPA, 1991c). The matrix interferences encountered in analysis of metals in tissue may 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.


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)

10.0 GUIDANCE FOR PERFORMING TIER II EVALUATIONS

10.1 Tier II: Water Column Effects

If a water column determination cannot be made in Tier I, the Tier II water column evaluation must be conducted for comparison with numeric water-quality standards (WQS) (Section 5.1). There are two approaches for the Tier II water column evaluation for WQS compliance. One approach is to use numerical models provided in Appendix C of this manual as a screen, assuming conservatively that all of the contaminants in the dredged material are released into the water column during the disposal process. The other approach 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 Relative To WQS

A screening approach may 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. The screen is not a requirement but is intended to reduce the effort required to develop information required for factual determinations.

Appendix C provides guidance on which numerical computer or analytical models should be applied to particular dredged material disposal projects and the information that is necessary to perform the evaluations. Versions of models for use on IBM-compatible microcomputers and example applications are provided on the diskettes in the pocket inside the back cover of this manual. The output of the appropriate 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 this contaminant is shown to meet the WQS, all of the other contaminants that require less dilution will also meet the WQS. The contaminant requiring the greatest dilution is determined by calculating the dilution that would be required to meet the WQS. To determine the dilution *D,* the following equation is solved for each contaminant of concern in terms of dissolved concentrations:

$$D = [(C_s \text{ x } SS/1000) — C_{wq}] / (C_{wq} — C_{ds})$$

where C_s = concentration of the contaminant in the dredged material expressed as micrograms per kilogram (μg/Kg), on a dry weight basis;

SS	=	suspended solids concentration in the dredged material discharge expressed as grams per liter (g/L);
1000	=	conversion factor, g to Kg;
C_{wq}	=	WQS in micrograms per liter (µg/L); and
C_{ds}	=	background concentration of the contaminant at the disposal site in micrograms per liter (µg/L).

Note that if the concentration of the constituent in the dredged material (C_s x SS/1000) is less than C_{wq}, no calculation is necessary since no dilution is required. Note also that, if the ambient disposal-site water concentration (C_{ds}) of a constituent is greater than C_{wq}, water quality at the disposal site cannot be met by dilution. Appendix C provides detailed information for performing the above calculations and identifying the contaminant of concern requiring the greatest dilution.

The concentration of this contaminant is then modeled to determine its maximum concentration in the water column outside the boundary of the mixing zone. If this concentration is below the applicable WQS, no additional testing is necessary to make a determination regarding WQS. If the concentration is higher, additional testing is necessary, as described in Section 10.1.2.

Note that 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, is necessary.

10.1.2 Elutriate Analysis Relative To WQS

For an elutriate analysis, the numerical mixing model (Appendix C) 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 provided in Section 9.4.2. The model is, in effect, using data that more accurately represent the contaminant concentrations that will be present in the water column after consideration of mixing. If the numerical model (Appendix C) predicts that the concentration of all contaminants of concern at the edge of the mixing zone is less than the available, applicable WQS, the dredged material complies with WQS. Otherwise, it does not.

10.1.2.1 Standard Elutriate Preparation

The standard elutriate test is used to predict the release of contaminants to the water column resulting from open water disposal. Prior to use, all labware should be thoroughly cleaned as appropriate for the contaminant analysis. At a minimum, labware should be washed with detergent, rinsed with acetone, 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. Enough elutriate should be prepared for the chemical analyses and for the water column toxicity tests in Tier III.

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 (22 ± 2°C). This is best accomplished by volumetric displacement. After the correct ratio is achieved, the mixture is stirred vigorously for 30 min with a mechanical or 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 without disturbing the settled material, and centrifuged to remove particulates prior to chemical analysis (approximately 2,000 rpm for 30 min, until visually clear). 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 provided in Section 9.4.2.

10.1.2.3 Comparison with WQS (Standard Elutriate Test)

The model need be run only for the contaminant that requires the greatest dilution to make a WQS determination. This contaminant may or may not be the same as that run in the screen (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. The contaminant requiring the greatest dilution is determined by calculating the dilution that would be required to meet the WQS. To determine the dilution D, the following equation is solved for each contaminant of concern in terms of dissolved concentrations:

$$D = (C_e - C_{wq})/(C_{wq} - C_{ds})$$

C_e = concentration of the dissolved contaminant in the standard elutriate in micrograms per liter (µg/L). All other terms are as previously defined in Section 10.1.1.

10.2 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 coarse screen to predict the magnitude of bioaccumulation likely to be associated with nonpolar organic contaminants in the dredged material. At present the TBP calculation can be performed only for nonpolar organic chemicals such as PCBs. However, methods for TBP calculations with metals and polar organic compounds are under development and may be added to this manual in the future. For the present, bioaccumulation potential of polar organic compounds, organometals, and metals in dredged material can only be tested (in Tiers III or IV), not calculated. However, it is still useful to calculate the TBP, which provides an indication of the magnitude of bioaccumulation of nonpolar organic compounds that may be encountered in testing at higher tiers. Additionally, if the TBP of the nonpolar organic compounds indicates that these contaminants are not bioavailable, this calculation may eliminate the need for further evaluation of these compounds and thereby reduce efforts in higher tiers.

Nonpolar organic chemicals include all organic compounds that do not dissociate or form ions. This includes the chlorinated hydrocarbon pesticides, many other halogenated hydrocarbons, PCBs, many PAHs including all the priority pollutant PAHs, dioxins and furans. It does not include metals and metal compounds, organic acids or salts, or organometallic complexes such as tributyltin or methyl mercury.

The environmental distribution 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). In organisms they are found primarily in the body fats or lipids (Konemann and van Leeuwen, 1980; Geyer et al., 1982; Mackay, 1982; Bierman, 1990). 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 TBP calculation 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. Another important assumption implicit in the TBP calculations is that there is no metabolic degradation or biotransformation of the chemical. Organic-carbon normalized contaminant concentrations are used such that the sediment-associated chemical can be characterized as 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. However, note that TBP calculations are not valid for sediments with TOC $\leq$ 0.2%.

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. The TBP calculation focuses 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 that the equilibrium concentration of nonpolar organic chemicals, which 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).

The possible chemical concentration 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 with PCBs, that a value of 4 for calculating LBP is appropriate. However, note that more precise values for specific chemicals are now available. Current information on such values may be obtained from the ACOE Contaminated Sediment Bulletin Board (BBS: phone number is 601-634-4380; settings are N, 8, 1). 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. Soft-bodied invertebrate lipid contents may range from 1 - 2% wet weight (based on data from an oligochaete, midge, and amphipod species [G. Ankley, EPA Duluth and H. Lee, EPA Newport, pers. comm.]).

Theoretical bioaccumulation potential (TBP) can be calculated relative to the biota sediment accumulation factor (BSAF) as

$$TBP = BSAF\ (C_s / \%TOC)\ \%L$$

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

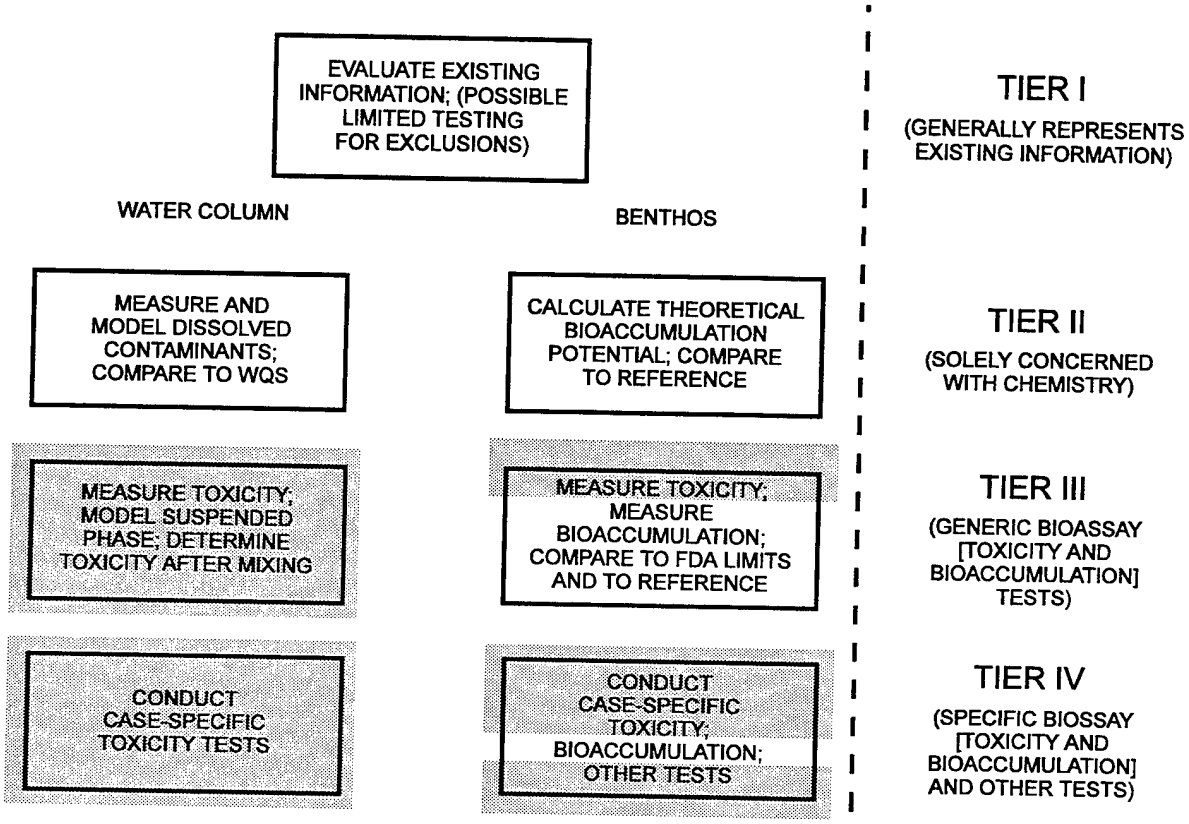
C_s = concentration of nonpolar organic chemical in the dredged material or reference sediment (any units of concentration may be used);

BSAF = 4 (Ankley et al., 1992c)

%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).


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)

11.0 GUIDANCE FOR PERFORMING BIOLOGICAL EFFECTS TESTS

Biological effects tests, i.e., toxicity tests, may be necessary if Tier I evaluations conclude that the dredged material contains contaminants which might result in an unacceptable adverse impact to the benthic environment and/or the water column. Toxicity tests with whole sediment are used to determine the potential for effects on benthic (bottom dwelling) organisms; toxicity tests with suspensions/solutions of dredged material are conducted to determine the potential effects on water column organisms.

The objective of water column toxicity tests is to determine the potential impact of dissolved and suspended contaminants on organisms in the water column, after considering mixing. Test organisms should be representative of appropriately sensitive water column species existing in the vicinity of the disposal site.

The objective of benthic toxicity tests 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 appropriately sensitive infaunal or epifaunal organisms existing in the vicinity of the disposal site. Benthic toxicity tests are intended to determine the potential chemical toxicity of a dredged material as distinct from its physical (e.g., grain-size) effects. Some organisms, particularly marine, are affected by differences in sediment textures or absence of sediments (McFarland, 1981; DeWitt et al., 1988). Control and reference sediments should be selected to minimize any artifactual effects of differences in grain size. If the sediment texture varies considerably between the dredged material and the control or reference sediments, any possible effects of grain size have to be determined and considered when designing the tests and evaluating the test results (e.g., DeWitt et al., 1988).

11.1 Tier III: Water Column Toxicity Tests

Tests to evaluate dredged-material impact on the water column involve 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 though some tests, e.g., bivalve larvae, may be run for shorter periods). The surviving organisms are examined at specified intervals and/or at the end of the test to determine if the test material is producing an effect. An introductory guide to general toxicity testing is presented in Part 8000 of APHA (1989) and in ASTM (1994b). Biological testing aspects of these reference publications may be followed as long as they do not conflict with this manual.

11.1.1 Species Selection

Three species are recommended for use in the water column exposure and should represent different phyla where possible (Table 11-1). The rationale for testing more than a single species is to cover the potential range of differing species sensitivities and to be environmentally protective. Of the species tested, at least one needs to be a sensitive benchmark (starred) species except as provided below; however, this does not preclude the use of more than one benchmark species. Those non-benchmark species listed in Table 11-1 or other species can be used if a summary of test conditions and test acceptability criteria similar to the starred benchmark species are established, and data from reference toxicity tests (see Appendix G.2.10.5.2) are provided on the sensitivity of the species. In order to be technically justfied, species proposed for use regionally and not listed in Table 11-1 would need to meet the species characteristics criteria, provided later in this Section, and proponents need to generate the following supporting information:

- data from toxicity tests using a set of reference chemicals with differing modes of action demonstrating that the proposed species is as sensitive or more sensitive than the species in Table 11-1

- summary of test conditions and test acceptability criteria.

If species proposed for use regionally are tested in conjunction with a benchmark species, the above supporting information is desirable but not needed. However, if the region substututes all species, the above information is needed.

The test organisms may be from healthy laboratory cultures or may be field collected, but not from 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 include, not in order of importance:

- readily available year-round
- tolerate handling and laboratory conditions
- give consistent, reproducible response to toxicants
- 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
- standardized test protocols are available
- can be readily tested as juveniles or larvae to increase sensitivity
- important ecologically, economically, and/or recreationally
- appropriately sensitive.

Table 11-1. Candidate Toxicity Test Species for Determining Potential Water Column Impact of Dredged Material Disposal. Details of testing procedures are provided in Appendix E.

Crustaceans

Mysid shrimp, *Mysidopsis* sp.* (N)[d]
*Neomysis americana** (N)
*Holmesimysis costata** (N)
Grass shrimp, *Palaemonetes* sp. (N)
Commercial shrimp, *Penaeus* sp. (N)
Cladocerans, *Daphnia magna** (F) [d]
*Daphnia pulex** (F) [d]
*Ceriodaphnia dubia** (F)[d]

Fish

Silversides, *Menidia* sp.* (N) (E)[d]
Sheepshead minnow,
*Cyprinodon variegatus** (N)[d]
Speckled sanddab, *Citharicthys stigmaeus* (N)
Grunion, *Leuresthes tenuis* (N)
Fathead minnow, *Pimephales promelas** (F)[d]
Bluegill sunfish, *Lepomis macrochirus* (F)
Channel catfish, *Ictalurus punctatus* (F)
Rainbow trout, *Oncorhynchus mykiss** (F)

Bivalves

Larvae of
Oyster, *Crassostrea* sp.* (N,E)[a]
Mussel, *Mytilus edulis** (N,E)[a]

Echinoderms

Larvae of
Sea urchins, *Strongylocentrotus* sp.*[bc] (N)
Lytechinus pictus[b] (N)
Sanddollar, *Dendraster* sp.*[bc] (N)

Note: Examples are not presented in order of importance; however, the asterisks indicate sensitive recommended benchmark species. Benchmark species comprise a substantial data base, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species. Other species may be designated in future as benchmark species by EPA and USACE when the data on their response to contaminants are adequate.

[a] fertilized egg to hinged, D-shaped prodissoconch I larvae. Note that these two species can be used in estuarine waters down to appropriate low levels of salinity (see Appendix E).
[b] fertilized egg to pluteus larvae
[c] sperm fertilization
[d] These species can also be used in sublethal, chronic testing (methods for such testing are available but not detailed in this manual).

For the purpose of this manual, related to the tolerances of the test animals, (F) = Freshwater, salinity ≤ 1 ‰ (N) = Near Coastal, salinity ≥ 25‰ (E) = Estuarine, salinity 1-25‰. It is recognized that the commonly accepted salinity range for estuaries is 1-35‰ and near coastal salinity is usually greater than 30‰ salinity.

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 echinoderms, crustaceans, molluscs, or fish are also appropriate organisms. Adult fish and molluscs and large crustaceans must not be used for water column toxicity testing because of their generally greater resistance to contaminants, except as additional test organisms where data on economically important species are necessary to address public or regional concerns.

Regardless of their source, test organisms should be collected and handled as gently as possible. They should be gradually acclimated to the test conditions if test conditions differ from holding conditions. Field collected organisms must be tested within 2 weeks of collection. Animals from established laboratory cultures can be held indefinitely. Further details on methods are provided in ASTM (1994b).

11.1.2 Apparatus

Water column toxicity tests are generally conducted as static exposures in pre-cleaned glass chambers equipped with covers to minimize evaporation. The size of the chambers depends on the size of the test species. Before use, all 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 with acetone, five times with tap water, and then thoroughly flushed with either distilled or deionized water.

Equipment and facilities must provide acceptable lighting requirements and temperature control. An environmental incubator or a water-bath system that allows temperature control within ±1°C is recommended.

11.1.3 Laboratory Conditions

Water column toxicity tests should be conducted under conditions known to be non-stressful to the test organisms. Salinity for marine/estuarine organisms should be stable within ±2‰ and, for all organisms, temperature should be stable within ±2°C throughout the exposure period. Dissolved-oxygen concentration should not be allowed to fall below an absolute minimum of 40% saturation for warm water species and 60% for cold water species. The temperature, salinity (if appropriate), dissolved oxygen, and pH in the test containers should be measured and recorded daily. Measurements of other parameters, for instance ammonia, may also be useful but need not be done daily.

11.1.4 Laboratory Procedures

Elutriate Preparation

Elutriate should be prepared using water collected from the dredging site. Disposal site water, clean seawater or freshwater, or artificial sea/salt mixtures should be used as dilution water for the tests. If sea/salt mixtures are used, they must be prepared in strict accordance with the manufacturer's instructions and allowed to age (with aeration) to ensure that all salts are in solution and pH has stabilized 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 volumetric ratio of 1:4 at room temperature (22 ± 2°C). The mixture is then stirred vigorously for 30 min with a mechanical or 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 for the organisms to be visible in the testing chamber. Note that 15-40 L of elutriate may need to be prepared to test some species.

Test Design

The number of replicate exposure chambers per treatment should be determined according to the guidance in Appendix E. A minimum of five replicates per treatment and 10 organisms (except zooplankton or larvae) per replicate is generally recommended. Organism loading density must be low enough to avoid overcrowding stress.

At least three concentrations of the dredged-material elutriate should be tested; recommended treatments are 100%, 50%, and 10%. Water from the same source in which the animals were held prior to testing must be included as a control treatment subject to test survival acceptability criteria for controls (Appendix G). To properly evaluate the test results, any toxicity at 100% dilution water should also be determined.

The test organisms should be approximately of equal size and/or age and assigned randomly to the different treatments. Zooplankton and larvae are usually transferred with the aid of a pipette. Air must not be trapped on or under the animals during the transfer process. Larger animals may be transferred in fine-mesh nets. Animals which are dropped or exhibit abnormal behavior should be discarded.

The test chambers should be covered and randomly placed in an incubator or water bath. The test type is static non-renewal; the control and test solutions are not replaced. During the exposure period, aeration should not be supplied (unless necessary to keep dissolved oxygen concentration above 40% saturation

for warm water species or 60% for cold water species), and the test solutions should not be stirred. Some species of crustaceans, particularly larval forms, may require feeding during the test. All food used must be analyzed to ensure that it is acceptably free of contaminants and will support survival, growth or reproduction of test organisms (cf. EPA, 1994b).

Recommended test duration is 48-96 h for zooplankton and some larvae (e.g., oysters) and up to 96 h for other organisms. For bivalve larvae, the ASTM (1994c) procedure should be used. Useful procedures for other organisms are given in ASTM (1994b). For some tests, intermediate time observations may be made of survival but, for other tests, survival is only assessed at the end of the testing period. For intermediate observations, care must be taken to minimize any stress to the test organisms. 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 intermediate observations, a pipette or forceps is used to remove dead organisms, molted exoskeletons, and food debris.

If greater than acceptable mean mortality or abnormal development occurs in the control as defined in the procedures for proper conduct of that test, the test must be repeated. Further QA/QC considerations are provided in Appendix G.

11.1.5 Data Presentation and Analysis

Data Presentation

The data for each test species should be presented in separate tables that include the following information:

- the scientific name of the test species
- the number of organisms in each treatment at the start of the test
- the number of organisms alive at each observation period, if applicable
- the number of organisms recovered alive and/or in normal health from each chamber at the end of the test
- additional information including water quality and any behavioral or other abnormalities.

Data Analysis

It is possible that no mortality or other effects will be observed in any of the treatments or that survival or other effects 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 water column toxicity attributable to the dredged material. However, if survival or other

effects in the dilution water treatment is at least 10% greater than the 100% dredged-material treatment, the data have to be evaluated statistically to determine whether the dredged-material suspension is significantly more toxic than the dilution water. If the 100% dredged-material treatment is not statistically different from the dilution water, the dredged material is predicted not to be acutely toxic to water column organisms. An LC_{50} should not be calculated unless at least 50% of the test organisms die in at least one of the serial dilutions . If there are no mortalities greater than 50%, then the LC_{50} is assumed to be ≥100%. If a statistical difference exists and greater than 50% mortality or other effects occur in all of the treatments, it is not possible to calculate an LC_{50} or EC_{50} value. If the conditions are highly toxic, such that the 10% treatment has greater than 50% mortality, further dilution must be made (new treatments of less than 10% dredged material) to attain a survival of greater than 50% and determine the LC_{50} or EC_{50} by interpolation. Statistical procedures recommended for analyzing the test data are described in detail in Appendix D.

11.1.6 Conclusions

The Tier III water-column effects evaluation involves using a numerical model comparison with the WQS. Descriptions of the models and applications are given in Appendix C, and the models are provided on the diskettes that can be found in the pocket inside the back cover of this manual.

The modeled concentrations of the dredged material (expressed as percentages) are compared to 0.01 of the 48- or 96-h LC_{50} or EC_{50}, depending on the test duration. The maximum allowable concentration outside the mixing zone is 0.01 LC_{50} or EC_{50}. Note that the 0.01 factor is intended for acute mortality data (e.g., relating acute to chronic toxicity) and not for more subtle effects such as abnormalities, growth or reproduction, including EC_{50} data (NAS, 1972). However, in the absence of other alternatives, the 0.01 application factor should be applied to EC_{50} data although it is recognized that these results will be conservative and that derivation of this historic application factor was largely a matter of "best professional judgement" by the NAS (1972). Thus, site-specific review may be required in some cases to determine compliance.

11.2 Tier III: Benthic Toxicity Tests

Toxicity tests with whole sediment are designed to determine whether the dredged material is likely to produce unacceptable adverse effects on benthic organisms. In benthic toxicity tests, the test animals are exposed to the whole sediment and any effects recorded.

11.2.1 Species Selection

Species representing three life history strategies are recommended for use in the whole sediment toxicity tests, one each representing a filter feeder, deposit feeder and a burrowing organism where possible (Table 11-2). The rationale for testing more than a single species is to cover the range of differing species sensitivities and to be environmentally protective. No single species is adequately protective of the broad range of possible chemical contaminants nor of the equally broad range of possible biological responses. Of the species tested, at least one sensitive benchmark (starred) species needs to be be used in all cases except as provided below; however, this does not preclude the use of benchmark species representative of all three required categories. If only two different species are being tested they should, together, cover the following three life history strategies: filter feeder, deposit feeder, burrower. Since amphipods are excellent organisms for short term toxicity, they are recommended as one of the species to be tested. Non-benchmark species listed in Table 11-2 can be used if a summary of test conditions and test acceptability criteria similar to the starred benchmark species are established and data from reference toxicity tests (see Appendix G.2.10.5.2) are provided on the sensitivity of the species. In order be technically justified, species proposed for use regionally and not listed in Table 11-2 need to meet the species characteristics criteria provided later in this section and proponents need to provide the following supporting information:

- data from toxicity tests using a set of reference chemicals with differing modes of action demonstrating that the proposed species is as sensitive or more sensitive than the species in Table 11-2

- summary of test conditions and test acceptability criteria.

If species proposed for use regionally are tested in conjunction with a benchmark species, the above supporting information is desirable but not required. However, if the region substitutes all species, the above information is needed.

Benthic organisms are used to evaluate the potential benthic impact of dredged material disposal. Testing of 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 and the Clean Water Act have shown that different species have various degrees of sensitivity to the physical and chemical composition of sediments.

To accurately evaluate potential benthic impact, appropriately sensitive toxicity test species should be related as closely as possible, both phylogenetically and ecologically, to benthic organisms in the disposal

Table 11-2. Candidate Acute Toxicity Test Species for Determining Potential Benthic Impact of Dredged-Material Disposal. Details of testing procedures are provided in Appendix E. Additional guidance is provided in ASTM (1994d,e,f,g) and EPA (1994c,d).

Amphipod Crustaceans

*Ampelisca abdita** (N)[a] [d,b]
*Rhepoxynius abronius** (N) [d,b]
Grandidierella japonica (N) [d,b]
Corophium sp. (N) [f,d,b]
*Leptocheirus plumulosus** (E,N)[a] [d,b]
*Eohaustorius estuarius** (E) [d,b]
*Hyalella azteca** (E,F)[a] [d,b]

Polychaetes

Neanthes arenaceodentata (N)[a] [d,b]

Juvenile Bivalves (clams)

Paper pondshell freshwater mussel, *Anodonta imbecillis* (F) [f,b]

Crustaceans other than Amphipods

Mysid shrimp, *Mysidopsis* sp. (N) [f,d]
Neomysis americana (N) [f]
Holmesimysis costata (N) [f]
Commercial shrimp, *Penaeus* sp. (N) [d,b]
Grass shrimp, *Palaemonetes* sp. (N,E)[b] [d]

Insect Larvae

Midges, *Chironomus tentans** (F)[a] [d,b]
*C. riparius** (F)[a] [d,b]
Mayfly, *Hexagenia limbata* (F) [d,b]

Oligochaetes

Pristina leidyi (F) [d,b]
Tubifex tubifex (F)[a] [d,b]
Lumbriculus variegatus (F)[a] [d,b]

Note: Examples are not presented in order of importance; however, the asterisks indicate sensitive recommended benchmark species. Benchmark species comprise a substantial data base, represent the sensitive range of a variety of ecosystems, and provide comparative data on the relative sensitivity of local test species. Other species may be designated in future as benchmark species by EPA and the USACE when the data on their response to contaminants are adequate. Only benthic species should be tested. Although sediment dwellers are preferable, intimate contact with sediment is acceptable. Note that testing with all recommended taxa is not required; however, at least one starred amphipod taxon must be tested.

[f = filter feeder; d = deposit feeder; b = burrower]. Note that *A. abdita*, *L. plumulosus*, *C. tentans*, and *H. limbata* are not direct filter feeders, but are suspension feeders.

[a] These species can also be used in sublethal, chronic testing (methods for such testing are available but not detailed in this manual).

[b] This species can be used in estuarine waters down to appropriate low levels of salinity (see Appendix E).

For the purposes of this manual, related to the tolerances of the test animals, (F) = Freshwater, salinity ≤ 1‰ (N) = Near Coastal, salinity ≥ 25‰ (E) = Estuarine, salinity 1-25‰. It is recognized that the commonly accepted salinity range for estuaries is 1-35‰ and near coastal water is usually greater than 30‰ salinity.

site area. Commercially important but possibly less sensitive benthic species in the vicinity of the disposal site may also be considered for testing.

Sediment grain size is likely to vary substantially between the dredged material, the reference sediment, and the control sediment. If candidate test species are overly sensitive to the different grain sizes (for instance, excessive mortality in the reference sediments attributable to grain size and not to other factors), either this must be taken into account (e.g., DeWitt et al., 1988) or other, more grain-size tolerant species should be considered for the project.

Final selection of test species for a particular dredged material disposal project should be made in consultation with regional regulatory and scientific personnel. Two phylogenetically and ecologically different species are recommended to account for different sensitivities to contaminants. The following is a list, not necessarily in order of importance, of characteristics to consider for species selection:

- readily available year-round
- preferably ingest sediments
- tolerate grain sizes of dredged material and control and reference sediments equally well or differences should be accounted for
- give consistent, reproducible response to toxicants
- tolerate handling and laboratory conditions
- related phylogenetically and/or by ecological requirements to species characteristic of the benthic environment of the disposal site area in the season of the proposed disposal
- standardized test protocols are available
- important ecologically, economically, and/or recreationally
- appropriately sensitive.

Infaunal amphipods are excellent organisms for short term toxicity tests with whole sediment (Swartz et al., 1979, 1985; Mearns and Word, 1982; Rogerson et al., 1985; Nebeker et al., 1984; Gentile et al., 1988; Scott and Redmond, 1989; Word et al., 1989; Burton, 1991), and are strongly recommended as appropriate test species for acute toxicity bioassays in marine/estuarine/fresh waters. Guidance on available testing procedures (static, 10-d exposures) provided in ASTM (1994d,e) may be followed on all points that do not conflict with this manual. Infaunal amphipods are:

- sensitive
- readily available
- as a group, tolerant of a wide range of grain sizes and laboratory exposure conditions
- ecologically relevant to most dredged material disposal sites.

The identity of all species should be verified by experienced taxonomists, particularly for animals collected in the field. If the toxicity test animals are also to be used in estimating bioaccumulation potential, the factors discussed in Section 12.1.1 for species selection should also be considered.

11.2.2 Laboratory Procedures

General Test Procedures

Acceptable water quality parameters during testing include but are not necessarily restricted to:

- the correct temperature and pH range
- adequate oxygen levels
- proper lighting
- the correct salinity range (near coastal and estuarine organisms)
- the correct hardness range (fresh water organisms)
- the absence of, or insignificant concentrations of, toxicants such as ammonia.

Amphipod and other small organism tests are often, but not always, conducted in 1 L containers under static conditions (Appendix E). Static renewal or even flow-through methods such as those described by Redmond et al. (1989) or Benoit et al. (1993) may be required for certain tests or where static non-renewal conditions would result in unacceptable build-up of, for instance, ammonia and/or sulfides (see second and third paragraphs, Ammonia and Sulfide toxicity, this section).

Before use, all glassware should be washed with detergent, rinsed with acetone, 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 must provide acceptable lighting requirements and temperature control. An environmental incubator or a water-bath system that allows temperature control within ± 1°C is recommended.

Dilution water should not be stressful to the test organisms, and should be stable throughout the exposure period. Salinity for marine/estuarine organisms should be stable within ± 2‰ and, for all organisms, temperature should be stable within ± 2°C throughout the exposure period. Dissolved oxygen concentration should not be allowed to fall below an absolute minimum of 40% saturation for warm water species and 60% for cold water species. The flow to the exposure chamber should be directed to achieve good mixing without disturbing the sediment on the bottom of the chamber.

A minimum of five replicate exposure chambers for the dredged material, reference, and control is recommended. The standard test duration is 10 d.

The quantity of sediment needed depends on the size of the exposure chambers. 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. Overcrowding of organisms must be avoided.

Prior to use in toxicity tests, sediments must be thoroughly homogenized. Very small amounts of clean diluent water may be added to facilitate mixing. If separation into liquid and solid phases occurs in posthomogenization storage, remixing will be required prior to usage.

The reference and control sediments, as well as the dredged material being tested, may contain live organisms. If necessary, macrobenthic organisms can be removed by press-sieving the sediments through an appropriately sized screen immediately prior to testing. The material remaining on the screen should be noted and discarded.

The experimental procedure described in ASTM (1994d) should be followed for preparing the exposure chambers for amphipod toxicity tests. For larger exposure chambers, sediment should be placed on the bottom of the exposure chamber and covered with clean diluent water; any sediment suspended during placement 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.

During the exposure period, daily records should be kept of obvious mortalities, emergence of infaunal organisms, formation of tubes or burrows, and any other or unusual behavior. Daily records of water quality (e.g., dissolved oxygen, salinity (if appropriate), ammonia, temperature, pH) should be maintained using test containers appropriate for this purpose. In flow-through or static-renewal systems, water quality may be kept within acceptable bounds by increasing the flow rate or frequency of water changes.

After the exposure period, live organisms are removed to clean diluent water, which may include sieving the sediments, and then counted. If greater than acceptable mean mortality occurs in the control, as defined in the procedures for proper conduct of that test, the test must be repeated. Organisms which show any response to gentle probing of sensitive parts or gentle swirling of the water should be considered alive. Sediment dwellers (e.g., amphipods) not recovered at the end of the test have to be considered dead. If organisms from these toxicity tests are to be used in estimating bioaccumulation potential, the survivors are gently and rapidly counted and then treated as described in Section 12.

Ammonia and Sulfide Toxicity

Whether ammonia is or is not a contaminant of concern depends on the disposal site. In order to identify elutriate or solid phase dredged material toxicity due to ammonia, it is essential to make routine measurements of ammonia on appropriate test fractions. These measurements are compared to water-only toxicity data for the same species used in the dredged material test (see Appendix F). The water-only toxicity data generated separately should be generated under conditions (e.g., pH, test length) reasonably similar to those in the test with the dredged material. If ammonia concentrations are too low to have potentially caused the observed toxicity in the dredged material sample, other contaminants are responsible for the toxicity. If ammonia concentrations are high enough to have caused the observed toxicity, toxicity identification evaluation (TIE) procedures should be used to confirm this suspicion. When there is no TIE confirmation that ammonia is responsible for sediment toxicity, it must be assumed that persistent contaminants other than ammonia are causing toxicity. Full details of procedures to identify ammonia as a toxicant in toxicity tests with dredged material are provided in Appendix F.

Whenever chemical evidence of ammonia is present at toxicologically important levels, i.e. ammonia concentrations exceed the species-specific acceptability ranges shown below (or 20 mg/L for freshwater organisms), and ammonia is not a contaminant of concern at the disposal site, the laboratory analyst should set up one or more beakers explicitly for the purpose of measuring interstitial ammonia. Ammonia in the sediment interstitial water should be reduced to below the species-specific level shown below (or to below 20 mg/L for freshwater organisms) before adding the benthic test organisms. Ammonia concentrations in the interstitial water can be reduced by sufficiently aerating the sample at saturation and replacing two volumes of water per day. The analyst should measure interstitial ammonia each day until it reaches a concentration below the appropriate species-specific level (or ≤20 mg/L for freshwater organisms). After placing the test organisms in the sediment, the analyst should ensure that ammonia concentrations remain within an acceptable range by conducting the toxicity test with continuous flow or volume replacement not to exceed two volumes per day. Peer-reviewed papers that deal with ammonia in sediments include: Dewitt et al. (1988), Scott and Redmond (1989), Burton (1991), EPA (1992, 1994c, 1994d), Benoit et al. (1993), Ankley et al. (1991, 1992a, 1992c, 1994).

General Acceptability Ranges for Ammonia in Marine and Estuarine Amphipod Sediment Toxicity Tests.

Parameter	*Rhepoxynius*	*Ampelisca*	*Eohaustorius*	*Leptocheirus*
Ammonia (total mg/L, pH 7.7)	<30	<30	<60	<60
Ammonia (unionized mg/L, pH 7.7)	<0.4	<0.4	<0.8	<0.8

The chemistry and toxicology of sulfides is less well-understood than that of ammonia. However, sulfides are not likely to be a problem in most open-water situations, or in bioassays where adequate oxygen levels are maintained in the overlying water.

11.2.3 Chronic/Sublethal Tests

Chronic/sublethal responses to sediment are presently only available, in addition to the end-point of survival, for a very few toxicity tests, for example: the amphipods *Hyalella azteca*, *Ampelisca abdita* and *Leptocheirus plumulosus;* the midges *Chironomus tentans* and *C. riparius*; the oligochaetes *Tubifex tubifex* and *Lumbriculus variegatus,* and the polychaete *Neanthes arenaceodentata.* [Note: EPA has recently developed chronic sediment toxicity test methods for freshwater organisms (*C. tentans and H. azteca*). EPA and USACE are jointly developing a chronic sediment toxicity test method manual for marine and estuarine organisms (*L. plumulosus*). These documents are currently under review and will be published as standard methods manuals.] Unlike acute toxicity tests, there is presently no consensus as to what level of chronic/sublethal effects (e.g., reduction of growth, reproduction, fecundity, survival of young) is cause for concern. Further, there is also no consensus as to when such effects would preclude disposal or would constitute unacceptable adverse effects requiring some type of management action. Hence, chronic/sublethal tests are not presently part of Tier III in this national manual. However, regional testing manuals may apply appropriate chronic/sublethal tests to sediments in advance of their inclusion in this national manual provided this is done with a benchmark species (e.g., *C. tentans*) or *in addition to* the benchmark testing.

Guidance for conducting the above tests may be found in publications including Nebeker and Miller (1988), Nebeker et al. (1984), Johns and Ginn (1990), Johns et al. (1990), Ingersoll and Nelson (1990), Dillon et al. (1993), Phipps et al. (1993), McGee et al. (1993). Burton (1991) provides a comprehensive review of freshwater sediment toxicity tests. Survival and growth are the endpoints of all of these tests. In addition, some tests also measure reproductive end-points.

Criteria for control acceptability for chronic/sublethal tests are specific to the test and organism. If control criteria are exceeded, the test must be repeated.

11.2.4 Data Presentation and Analysis

Data Presentation

The data for each test species should be presented in separate tables that include the following information:

- scientific name of the test species

- number of organisms in each treatment at the start of the test
- number of organisms recovered alive and/or in normal health from each chamber at the end of the test (including positive and negative controls)
- information regarding emergence, burrowing, tube building, behavioral abnormalities, growth, reproduction, and any other observations
- water-quality data for each test chamber for each day.

Data Analysis

It is possible that neither mortality nor other effects will be observed in any of the treatments or that survival in the dredged material will be equal to or higher than survival in the reference or control sediments. In either of these situations, there is no need for statistical analysis and no indication of adverse effects due to the dredged material. Similarly, if survival is higher in test sediments than in the control, but lower than in the reference area, and control survival is at acceptable levels (i.e., 90% or greater survival), there is no need for statistical analysis and no indication of benthic toxicity due to the dredged material. However, if survival in the reference sediment is higher than in the dredged material treatments and exceeds the allowable percent difference between the two treatments, the data have to be analyzed statistically to determine whether there is a significant difference between the reference and dredged material. Statistical procedures recommended for analyzing benthic acute toxicity data are described in detail in Appendix D. Local guidance must be developed to interpret chronic/sublethal tests.

11.2.5 Conclusions

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

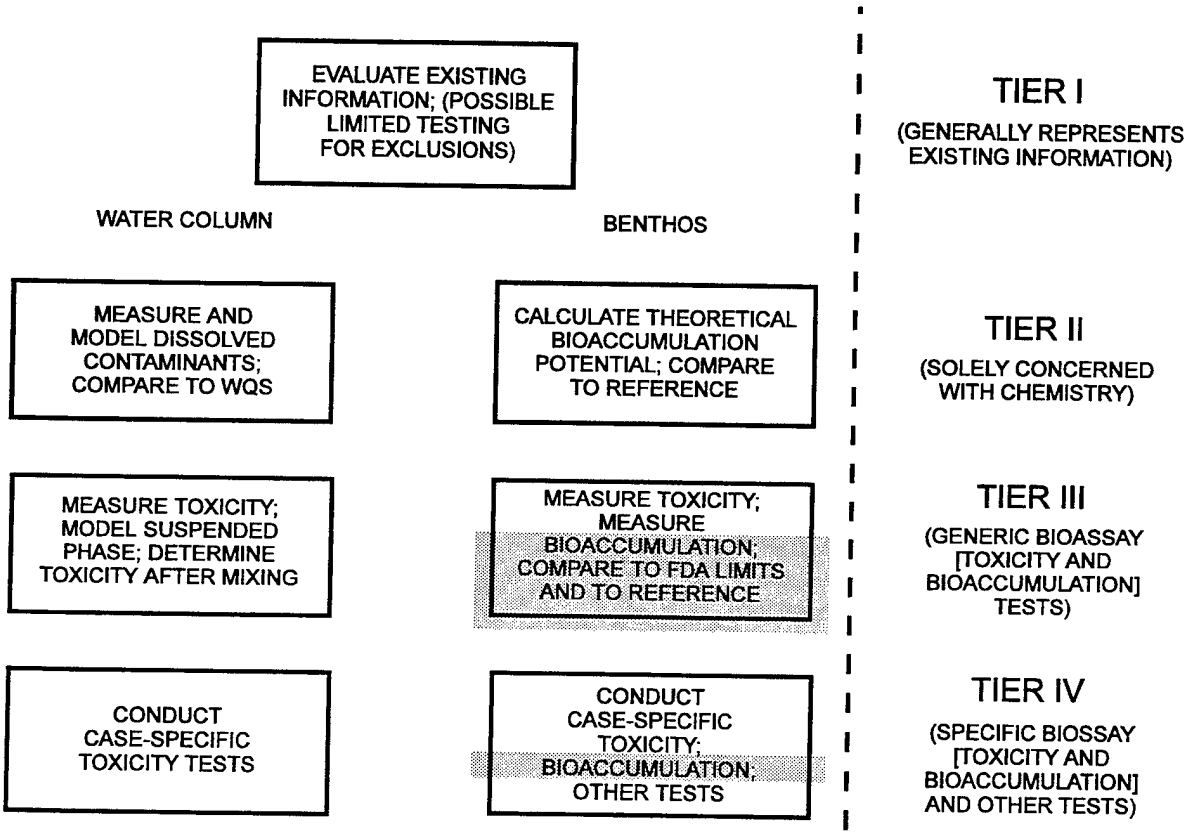
11.3 Tier IV: Chronic/Sublethal Effects Evaluations

At present, it is not appropriate to incorporate sediment chronic/sublethal effects testing in this national manual (see Sections 6.0 and 11.2.3). When standardized chronic effects tests are approved, they will be incorporated in Tier III. Until then, such non-standard tests should be used in Tier IV except where regional testing manuals apply such tests in advance of their inclusion in future revisions of this national manual, provided this is done with a benchmark species or *in addition to* the benchmark testing.

11.4 Tier IV: Case Specific Evaluations

Biological effects tests in Tier IV should be used only in situations that warrant special investigative procedures. They may include chronic/sublethal tests, field studies such as benthic infaunal studies (EPA, 1992), experimental studies such as *in situ* toxicity tests or toxicity identification evaluation (Ankley et

al., 1992a), risk assessments and/or no effects levels for aquatic life. In such cases, test procedures have to be tailored for specific situations, and general guidance cannot be offered. Such studies have to be selected, designed, and evaluated as the need arises, with the assistance of administrative and scientific expertise from EPA and USACE, and other sources as appropriate.


EVALUATE EXISTING INFORMATION; (POSSIBLE LIMITED TESTING FOR EXCLUSIONS)
TIER I
(GENERALLY REPRESENTS EXISTING INFORMATION)
WATER COLUMN
BENTHOS
MEASURE AND MODEL DISSOLVED CONTAMINANTS; COMPARE TO WQS
CALCULATE THEORETICAL BIOACCUMULATION POTENTIAL; COMPARE TO REFERENCE
TIER II
(SOLELY CONCERNED WITH CHEMISTRY)
MEASURE TOXICITY; MODEL SUSPENDED PHASE; DETERMINE TOXICITY AFTER MIXING
MEASURE TOXICITY; MEASURE BIOACCUMULATION; COMPARE TO FDA LIMITS AND TO REFERENCE
TIER III
(GENERIC BIOASSAY [TOXICITY AND BIOACCUMULATION] TESTS)
CONDUCT CASE-SPECIFIC TOXICITY TESTS
CONDUCT CASE-SPECIFIC TOXICITY; BIOACCUMULATION; OTHER TESTS
TIER IV
(SPECIFIC BIOSSAY [TOXICITY AND BIOACCUMULATION] AND OTHER TESTS)

12.0 GUIDANCE FOR PERFORMING BIOACCUMULATION TESTS

Bioaccumulation is defined in relation to disposal activities in the Definitions section at the beginning of this manual.

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. Lee et al. (1989) and Boese and Lee (1992) discuss 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 28 d of exposure (see Section 6.3). Although time series testing is a component of Tier IV bioaccumulation testing, it may also be appropriate in Tier III, for instance where K_{ow} values are greater than 5.5 (see Section 12.2.1).

12.1.1 Species Selection and Apparatus

The selection of aquatic organisms for use in the determination of bioaccumulation will depend on their inability to metabolize some types of organic compounds, and their ability to survive exposure to the test sediments. Two species should be used in bioaccumulation testing where possible (Table 12-1), unless adequate regional data are available to justify single species testing. Test species should provide adequate biomass for chemical analysis, and preferably ingest sediments and survive in dredged material and control and reference sediments equally well (or where differences can be accounted for). The rationale for testing more than a single species is to cover the range of differing species contaminant accumulation and to be environmentally protective. Of the species tested, at least one must be a benchmark species; however, this does not preclude the use of more than one benchmark species. Non-benchmark species listed in Table 12-1 can achieve benchmark status if a summary of test conditions and test acceptability criteria similar to the starred benchmark species are provided that meet the required species characteristics criteria. To be technically justified, species proposed for use regionally and not listed in Table 12-1 would also need to meet the species characteristics criteria and proponents should provide a summary of test conditions and test acceptability criteria except where species are to be tested *in addition to* the benchmark species. In this latter case, this information is desirable but not needed.

Table 12-1. Candidate Test Species for Determining Potential Bioaccumulation from Whole Sediment Tests. Details of testing procedures are provided in Appendix E; additional guidance is provided in EPA (1994c,d).

Polychaetes

*Neanthes arenaceodentata** (N)
*Nereis virens** (N,E)[a]
Arenicola marina (N)

Oligochaetes

Lumbriculus variegatus (F)*

Insect Larvae

Mayfly, *Hexagenia limbata* or sp. (F)

Bivalves

Macoma clam, *Macoma nasuta**(N,E)[a]
Yoldia clam, *Yoldia limatula* (N)

Crustaceans

Diporeia sp. (F)

Note: Examples are not presented in order of importance; however, the asterisks indicate recommended benchmark species. Other species may be designated in future as benchmark species by EPA and USACE when the data on their response to contaminants are adequate. Only benthic species should be tested. Although sediment ingesters are preferable, intimate contact with sediment is acceptable.

Only tests which do not require feeding of the organisms are included. Feeding is a research issue; for the present, food is not to be added because it provides additional organic carbon and can alter contaminant partitioning during testing.

For the purpose of this manual, related to the tolerances of the test animals, (F) = Freshwater, salinity ≤ 1‰ (N) = Near Coastal, salinity ≥ 25‰ (E) = Estuarine, salinity 1-25‰. It is recognized that the commonly accepted salinity range for estuaries is 1-35‰ and near coastal water is usually greater than 30‰ salinity.

[a] *Macoma nasuta* and *Nereis virens* bioaccumulation tests are in the process of standardization by EPA; it is expected that these will, in future, be the primary benchmark species for near coastal waters. Further, these two species can be used in estuarine waters down to appropriate low levels of salinity (see Appendix E).

Apparatus to be used for testing is described in Section 11.2.2. Additional requirements for voiding gut contents are described in Section 12.1.2. Species characteristics to consider when designing bio-accumulation tests include, not in order of importance:

- readily available year-round
- provide adequate biomass for analysis
- preferably ingest sediments
- preferably high in lipids
- survive in dredged material and control and reference sediments equally well, allowing adequate tissue for analysis
- tolerate handling and laboratory conditions
- related phylogenetically and/or by ecological requirements to species characteristic of the disposal site area in the season of the proposed discharge
- important ecologically, economically, and/or recreationally
- inefficient metabolizers of contaminants, particularly PAH.

Regional scientists and regulatory personnel should be consulted for additional guidance. A minimum amount of tissue is required for analysis, otherwise it will be impossible to quantify the amount of contaminant present (Section 9.5.2). Examples of the amounts of tissue which may be required are provided in Table 8-2. However, the amounts shown are not set amounts; more or less may be required depending on the analytes, matrices, detection limits, and particular analytical laboratory. If the biological needs of the organisms or adequate voiding (e.g., clams) require the presence of sediment, uncontaminated sand should be used (Section 12.1.2). Data in the form of "concentration below detection limits" are not quantitative; definitive concentration measurements are the goal, where such are possible within reasonable method and target detection limits.

12.1.2 Experimental Conditions

Test conditions are similar but not identical to those described in Section 11.2.2 for whole sediment toxicity tests. Overlying water renewal may be required to maintain adequate water quality. Food or additional sediment should not be provided during the test. Control animals should be sampled and archived at both the beginning and the end of testing. If discrepancies are found during data analysis, the archived samples can be analyzed to possibly resolve any problem(s). Due care should be taken not to exceed species-specific biomass loadings (overcrowding; APHA, 1989).

Digestive tracts of the animals should be emptied or removed immediately after termination of the exposure period. Sediment in digestive tracts may contain inert constituents and the contaminants of concern in forms which are not biologically available but which may be incorrectly identified as such during chemical analysis (e.g., see Lobel et al., 1991).

If the animals are large enough to make it practical, the best procedure is to excise the digestive tract. However, test organisms are seldom large enough to allow this, and most organisms have to be allowed to void the material, in separate aquaria in clean, sediment-free water. Some organisms 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 tissues, purging for longer periods is not recommended. Shells or exoskeletons which generally contain low levels of contaminants are, where possible, removed and not included in the analysis as their weight would give an artificially low indication of bioavailability.

An initial time-zero of each sample is collected for tissue analysis. Tissue contaminant concentrations in control animals must be determined to ensure that background levels are not inordinate. Although procedures for Tier III and IV laboratory bioaccumulation tests have been discussed separately, it may be possible to combine these procedures in practice. This can be done by following the steady state (Tier IV) bioaccumulation procedure which involves sequential time-series analyses, but initially analyzing only the 28 d sample and freezing the other samples. If these data, as part of the Tier III bioavailability evaluation, do not allow a determination to be made, then the remaining time series samples may be analyzed and used in the Tier IV steady-state bioaccumulation evaluation.

12.1.3 Chemical Analysis

Chemical analysis will involve some or all of the contaminants identified in Sections 4.2 and 9.5.1. Analytical procedures are provided in Section 9.5.2.

12.1.4 Data Presentation and Analysis

Data Presentation

Data should be presented in tabular format, listing tissue concentration of each contaminant, by organism and by sediment type (e.g., dredged and reference). Similar information to that detailed in Section 11.2.4

should be provided. Although bioaccumulation species/tests cannot be used to determine toxicity requirements, any mortalities which occur during bioaccumulation testing must be documented.

Data Analysis

Contaminant tissue concentrations in test organisms are statistically compared to the FDA Action Levels (Table 6-1) (refer to Figure 3-3). These tissue concentrations are also statistically compared with reference organism concentrations (Appendix D). In some cases, tissue concentrations in organisms exposed to one or more of the dredged-material samples may be less than or equal to reference organism concentrations. Providing the reference data are appropriate, this result indicates that bioavailability of the contaminants of concern in the dredged material is not greater than in the reference area sediment.

The sample of organisms archived 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. In some cases, elevated body burdens may not be due to the dredged material or reference sediment, but may have been already present in the organisms at the start of the test.

12.1.5 Conclusions

Guidance on reaching a determination is provided in Section 6.3.

12.2 Tier IV: Determination Of Steady State Bioaccumulation

Tier IV bioaccumulation evaluation, if necessary, provides for determination, either by laboratory testing (ASTM, 1984) or by collection of field samples, of the steady state concentrations of contaminants in organisms exposed to the dredged material as compared with organisms exposed to the reference site material. Testing options include longer laboratory exposures (not discussed), collection of organisms living in the material to be dredged and at the reference site for body burden determinations (Section 12.2.2) or *in situ* exposures using transplanted organisms, for instance caged mussels (not discussed). Tier IV determinations follow the guidance in Section 7.2.

12.2.1 Laboratory Testing

The necessary species, apparatus and test conditions for laboratory testing are those for Tier III bioaccumulation testing (see Sections 12.1.1 and 12.1.2). Tissue samples taken at different times during

the exposure period provide the basis for determining the rate of uptake and elimination of contaminants. 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. For the purposes of this test, steady state is defined as the concentration of contaminant that would occur in tissue after constant exposure conditions.

An initial time-zero sample of each species is collected for tissue analysis. Additional tissue samples are collected from each of the five replicate reference and dredged-material exposure chambers at intervals of, for instance, 2, 4, 7, 10, 18, and 28 d. It is critical that enough tissue is available to allow for interval body burden analyses at the specified detection limits.

Complete tissue concentration data should be presented in tabular format. Recommended statistical methods for fitting a curve to determine steady-state tissue concentration are provided in Appendix D. The statistical procedures use an iterative curve-fitting process to determine the key variables ($k_1 C_s$ the uptake rate-constant times the contaminant concentration in the sediment, and k_2 the depuration rate constant). An initial value for C_s has to be supplied. When the sediment concentration of the contaminant of concern is used, the ratio of k_1/k_2 is the sediment bioaccumulation factor (BAF) (Lake et al., 1987; Rubinstein et al., 1987), the ratio of steady-state tissue concentration to sediment concentration.

A determination is made based on the magnitude of bioaccumulation from the dredged material, its comparison with the available FDA levels, steady-state bioaccumulation from the reference sediment, and the body burden of reference organisms. Guidance for making determinations based on these comparisons is provided in Section 7.2 and can include risk assessment and no effects levels for aquatic life.

Guidance on quality assurance/quality control (QA/QC) considerations for bioaccumulation testing are provided in Appendix G.3.17 and EPA (1995).

12.2.2 Field Assessment of Steady State Bioaccumulation

Field sampling programs obviate 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. Difficulties related to the time required to conduct laboratory bioaccumulation studies are also overcome if organisms already living at the disposal site are used for field bioaccumulation studies. This approach is technically valid for predictive purposes only where there is a true historical precedent for the proposed operation being evaluated. That is, a field assessment can be used only 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, 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. This approach is generally not appropriate for multi-user disposal sites. 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

Major items required include:

- a vessel capable of operating at the disposal site and equipped to handle benthic sampling devices; navigation equipment has to allow precise positioning
- sampling devices such as a box corer, Smith-MacIntyre, Van Veen, Petersen, Ponar, Ekman or other benthic grab
- stainless steel screens 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 adequate sample collection at all stations and to provide sufficient tissue for analysis (see Section 12.1.1). The same species must be collected at all stations because bioaccumulation cannot be compared across species lines. If these conditions cannot be met, the field assessment approach cannot be implemented.

If possible, several samples of sufficient size for analysis should be collected at each station to provide a statistical estimate of variability in tissue contaminant content. Collection of more than one sample per station, however, may prove impractical if a composite of many small organisms has 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 crustaceans, certain molluscs, and fish) should not be used, because a relationship cannot be established between their location when collected and their

body burden at the time of collection. Therefore, relatively large, immobile species are the most desirable organisms. However, analyses should not be conducted on single organisms as the objective is to obtain representative data for the entire population of 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 previously, if PAH are contaminants of concern, it is essential that bioaccumulation studies include one or more species with very low ability to metabolize PAH. Bivalve molluscs and oligochaetes are widely accepted as meeting this requirement.

12.2.2.3 Sampling Design and Conduct

Sufficient tissue to obtain definitive body burden data has to be collected using the same species from each of at least three stations within the disposal site boundaries and from an acceptable reference site. It is mandatory that several stations be sampled, rather than collecting all of the animals at one station, in order to 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.

12.2.2.4 Basis for Evaluation of Bioaccumulation

Evaluations are made by comparison to contaminant concentrations in field organisms living around, but not affected by, the disposal site, similar to the reference area approach (Section 3.1). In this case, reference data involve at least three stations located in an uncontaminated material sedimentologically similar to that within the disposal site, in a direction perpendicular to (i.e., not in the direction of) the net bottom transport. 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.

12.2.2.5 Sample Collection and Handling

Repeated collections should be made at the same location 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.

Label the samples clearly and return the organisms to the laboratory, being careful to keep them separated and to maintain nonstressful levels of temperature and dissolved oxygen. In the laboratory, maintain them in clean water in separate containers. Do not place any sediment in the containers and do not feed the organisms. Immediately discard any organisms that die. Remove sediment from the digestive tracts of the organisms and, as possible, shells or exoskeletons (Section 12.1.2).

12.2.2.6 Chemical Analysis

Chemical analysis will involve some or all of the contaminants identified in Sections 4.2 and 9.5.1. Analytical procedures are provided in Section 9.5.2.

12.2.2.7 Data Presentation and Analysis

Complete tissue concentration data for all samples should be presented in tabular format as previously described. Since Tier IV testing will generally use non-standard methods and approaches, complete documentation is critical. Recommended statistical methods presented in Appendix D may not include all data analyses necessary for all Tier IV tests.

12.2.2.8 Conclusions

A determination is made based on the magnitude of bioaccumulation in organisms collected within the boundaries of the reference site, compared with bioaccumulation in organisms living within the area to be dredged. Guidance for making a determination based on these comparisons is provided in Section 7.2.

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APPENDIX A
40 CFR PART 230*

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ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 230

Guidelines for Specification of Disposal Sites for Dredged or Fill Material

[WH-FRL 1647-7]

45 FR 85336

December 24, 1980

AGENCY: Environmental Protection Agency.

ACTION: Rule.

SUMMARY: The 404(b)(1) Guidelines are the substantive criteria used in evaluating discharges of dredged or fill material under section 404 of the Clean Water Act. These Guidelines revise and clarify the September 5, 1975 Interim final Guidelines regarding discharge of dredged or fill material into waters of the United States in order to:

(1) Reflect the 1977 Amendments of Section 404 of the Clean Water Act (CWA);

(2) Correct inadequacies in the interim final Guidelines by filling gaps in explanations of unacceptable adverse impacts on aquatic ecosystems and by requiring documentation of compliance with the Guidelines; and

(3) Produce a final rulemaking document.

EFFECTIVE DATE: These Guidelines will apply to all 404 permit decisions made after March 23, 1981. In the case of civil works projects of the United States Army Corps of Engineers involving the discharge of dredged or fill material for which there is no permit application or permit as such, these Guidelines will apply to all projects on which construction or dredging contracts are issued, or on which dredging is initiated for Corps operations not performed under contract, after October 1, 1981. In the case of Federal construction projects meeting the criteria in section 404(r), these Guidelines will apply to all projects for which a final environmental impact statement is filed with EPA after April 1, 1981.

FOR FURTHER INFORMATION CONTACT: Joseph Krivak, Director, Criteria and Standards Division (WH-585), Environmental Protection Agency, 401 M Street, S.W., Washington, D.C. 20460, telephone (202) 755-0100.

SUPPLEMENTARY INFORMATION:

Background

The section 404 program for the evaluation of permits for the discharge of dredged or fill material was originally enacted as part of the Federal Water Pollution Control Amendments of 1972. The section authorized the Secretary of the Army acting through the Chief of Engineers to issue permits specifying disposal sites in accordance with the section 404(b)(1) Guidelines. Section 404(b)(2) allowed the

Secretary to issue permits otherwise prohibited by the Guidelines, based on consideration of the economics of anchorage and navigation. Section 404(c) authorized the Administrator of the Environmental Protection Agency to prohibit or withdraw the specification of a site, upon a determination that use of the site would have an unacceptable adverse effect on municipal water supplies, shellfish beds and fishery areas (including spawning and breeding areas), wildlife, or recreational areas.

Under section 404(b)(1), the Guidelines are to be based on criteria comparable to those in section 403(c) of the Act, for the territorial seas, contiguous zone, and oceans. Unlike 403(c), 404 applies to all waters of the United States. Characteristics of waters of the United States vary greatly, both from region to region and within a region. There is a wide range of size, flow, substrate, water quality, and use. In addition, the materials to be discharged, the methods of discharge, and the activities associated with the discharge also vary widely. These and other variations make it unrealistic at this time to arrive at numerical criteria or standards for toxic or hazardous substances to be applied on a nationwide basis. The susceptibility of the aquatic ecosystem to degradation by purely physical placement of dredged or fill material further complicates the problem of arriving at nationwide standards. As a result, the Guidelines concentrate on specifying the tools to be used in evaluating and testing the impact of dredged or fill material discharges on waters of the United States rather than on simply listing numerical pass-fail points.

The first section 404(b)(1) Guidelines were promulgated by the Administrator in interim final form on September 5, 1975, after consultation with the Corps of Engineers. Since promulgation of the interim final Guidelines, the Act has been substantially amended. The Clean Water Act of 1977 established a procedure for transferring certain permitting authorities to the states, exempted certain discharges from any section 404 permit requirements, and gave the Corps enforcement authority. These amendements also increased the importance of the section 404(b)(1) Guidelines, since some of the exemptions are based on alternative ways of applying the Guidelines. These changes, plus the experience of EPA and the Corps in working with the interim final Guidelines, have prompted a revision of the Guidelines. The proposed revision attempted to reorganize the Guidelines, to make it clearer what had to be considered in evaluating a discharge and what weight should be given to such considerations. The proposed revision also tightened up the requirements for the permitting authority's documentation of the application of the Guidelines.

After extensive consultation with the Corps, the proposed revisions were put out for public comment (44 FR 54222, September 18, 1979). EPA has reviewed, and, after additional consultation with the Corps, revised the proposal in light of these comments. This preamble addresses the significant comments received, explains the changes made in the regulation, and attempts to clear up some misunderstandings which were revealed by the comments. Response to Significant Comments

Regulation Versus Guideline

A number of commenters objected to the proposed Guidelines on the grounds that they were too "regulatory." These commenters argued that the term "guidelines" which appears in section 404(b)(1) requires a document with less binding effect than a regulation. EPA disagrees. The Clean Water Act does not use the word "guideline" to distinguish advisory information from regulatory requirements. Section 404(b)(2) clearly demonstrates that Congress contemplated that discharges could be "prohibited" by the Guidelines. Section 403 (which is a model for the 404 (b)(1) Guidelines) also provides for "guidelines" which are clearly regulatory in nature. Consequently, we have not changed the regulation to make it simply advisory. Of course, as the regulation itself makes clear, a certain amount of flexibility is still intended. For example, while the ultimate conditions of compliance are "regulatory", the Guidelines allow some room for judgment in determining what must be done to

arrive at a conclusion that those conditions have or have not been met. See, for example, @ 230.6 and Sec. 230.60, and introductory sentence in Sec. 230.10.

Statutory Scheme and How the Guidelines Fit Into It

A number of commenters with objections appeared confused about EPA's role in the section 404 program. Some wondered why EPA was issuing Guidelines since EPA could stop an unacceptable discharge under section 404(c). Others were uncertain how the Guidelines related to other section 404 regulations.

The Clean Water Act prohibits the discharge of dredged or fill material except in compliance with section 404. Section 404 sets up a procedure for issuing permits specifying discharge sites. Certain discharges (e.g. emergency repairs, certain farm and forest roads, and other discharges identified in sections 404(f) and (r)) are exempted from the permit requirements. The permitting authority (either the Corps of Engineers or an approved State program) approves discharges at particular sites through application of the section 404(b)(1) Guidelines, which are the substantive criteria for dredged and fill material discharges under the Clean Water Act. The Corps also conducts a Public Interest Review, which ensures that the discharge will comply with the applicable requirements of other statutes and be in the public interest. The Corps or the State, as the case may be, must provide an opportunity for a public hearing before making its decision whether to approve or deny. If the Corps concludes that the discharge does not comply with the Guidelines, it may still issue the permit under 404(b)(2) if it concludes that the economics of navigation and anchorage warrant. Section 404(b)(2) gives the Secretary a limited authority to issue permits prohibited by the Guidelines; it does not, as some commenters suggested, require the Guidelines to consider the economics of navigation and anchorage. Conversely, because of 404(b)(2), the fact that a discharge of dredged material does not comply with the Guidelines does not mean that it can never be permitted. The Act recognizes the concerns of ports in section 404(b)(2), not 404(b)(1). Many readers apparently misunderstood this point.

EPA's role under section 404 is several-fold. First, EPA has the responsibility for developing the 404(b)(1) Guidelines in conjunction with the Corps. Second, EPA reviews permit applications and gives its comments (if any) to the permitting authority. The Corps may issue a permit even if EPA comments adversely, after consultation takes place. In the case of state programs, the State director may not issue a permit over EPA's unresolved objection. Third, EPA has the responsibility for approving and overseeing State 404 programs. In addition, EPA has enforcement responsibilities under section 309. Finally, under either the Federal or State program, the Administrator may also prohibit the specification of a discharge site, or restrict its use, by following the procedures set out in section 404(c), if he determines that discharge would have an unacceptable adverse effect on fish and shellfish areas (including spawning and breeding areas), municipal water supplies, wildlife or recreation areas. He may do so in advance of a planned discharge or while a permit application is being evaluated or even, in unusual circumstances, after issuance of a permit. (See preamble to 40 CFR Part 231, 44 FR 58076, October 9, 1979.) If the Administrator uses 404(c), he may block the issuance of a permit by the Corps or a State 404 program. Where the Administrator has exercised his section 404(c) authority to prohibit, withhold, or restrict the specification of a site for disposal, his action may not be overridden under section 404(b)(2). The fact that EPA has 404(c) authority does not lessen EPA's responsibility for developing the 404(b)(1) Guidelines for use by the permitting authority. Indeed, if the Guidelines are properly applied, EPA will rarely have to use its 404(c) veto.

The Clean Water Act provides for several uses of the Guidelines in addition to the individual permit application review process described above. For example, the Corps or an approved state may issue General permits for a category of similar activities where it determines, on the basis of the 404(b)(1) Guidelines, that the activities will cause only minimal adverse environmental effects both individually

and cumulatively (Section 404(e) and (g)(1)). In addition, some of the exemptions from the permit requirements involve application of the Guidelines. Section 404(r) exempts discharges associated with Federal construction projects where, among other things, there is an Environmental Impact Statement which considers the 404(b)(1) Guidelines. Section 404(f)(1)(F) exempts discharges covered by best management practices (BMP's) approved under section 208(b)(4)(B) and (c), the approval of which is based in part on consistency with the 404(b)(1) Guidelines.

Several commenters asked for a statement on the applicability of the Guidelines to enforcement procedures. Under sections 309, 404(h)(1)(G), and 404(s), EPA, approved States, and the Corps all play a role in enforcing the section 404 permit requirements. Enforcement actions are appropriate when someone is discharging dredged or fill material without a required permit, or violates the terms and conditions of a permit. The Guidelines as such are generally irrelevant to a determination of either kind of violation, although they may represent the basis for particular permit conditions which are violated. Under the Corps' procedural regulations, the Corps may accept an application for an after-the-fact permit, in lieu of immediately commencing an enforcement action. Such after-the-fact permits may be issued only if they comply with the 404(b)(1) Guidelines as well as other requirements set out in the Corps' regulations. Criteria and procedures for exercising the various enforcement options are outside the scope of the section 404(b)(1) Guidelines.

Some commenters suggested that we either include specific permit processing procedures or that we cross-reference regulations containing them. Such procedures are described in 33 CFR Part 320-327 (Corps' procedures) and in 40 CFR Part 122-124 (minimum State procedures). When specific State 404 programs are approved, their regulations should also be consulted.

How Future Changes in the Testing Provision Relate to Promulgation of This Final Rule

The September 18, 1979, proposal contained testing provisions which were essentially the same as those in the Interim Final regulations. The Preamble to that proposal explained that it was our intention to propose changes in the testing provisions, but that a proposal was not yet ready. Consequently, while we have been revising the rest of the Guidelines, we have also been working on a proposal for reorganizing and updating the testing provisions. Now that we have finalized the rest of the Guidelines, two options are available to us. First, we could delay issuing any final revisions to our 1979 proposal until we could propose a revised testing package, consider comments on it, and finalize the testing provisions. We could then put together the Guidelines and the revised testing section in one final regulation. The 1975 interim final Guidelines would apply in their entirety until then. Second, we could publish the final Guidelines (with the 1975 testing provisions) and simultaneously propose changes to the testing provision. It is our present belief that proposed changes to the testing provision would not affect the rest of the Guidelines, but the public would be allowed to comment on any inconsistencies it saw between the rest of the Guidelines and the testing proposal. Then, when the comments to the testing proposal had been considered, we would issue a new final regulation incorporating both the previously promulgated final Guidelines and the final revised testing provision.

We have selected the second option because this approach ensures that needed improvements to the Guidelines are made effective at the earliest possible date, it gives the public ample opportunity to comment on the revised testing section, and it maintains the 1975 testing requirements in effect during the interim which would be the case in any event.

Guideline Organization

Many readers objected to the length and complexity of the Guidelines. We have substantially reorganized the regulation to eliminate duplicative material and to provide a more logical sequence.

These changes should make it easier for applicants to understand the criteria and for State and Corps permit evaluators and the Administrator to apply the criteria. Throughout the document, we have also made numerous minor language changes to improve the clarity of the regulations, often at the suggestion of commenters.

Following general introductory material and the actual compliance requirements, the regulations are now organized to more closely follow the steps the permitting authority will take in arriving at his ultimate decision on compliance with the Guidelines.

By reorganizing the Guidelines in this fashion, we were also able to identify and eliminate duplicative material. For example, the proposed Guidelines listed ways to minimize impacts in many separate sections. Since there was substantial overlap in the specific methods suggested in those sections, we consolidated them into new Subpart H. Other individual sections have been made more concise. In addition, we have decreased the number of comments, moving them to the Preamble or making them part of the Regulation, as appropriate.

General Permits

When issued after proper consideration of the Guidelines, General permits are a useful tool in protecting the environment with a minimum of red tape and delay. We expect that their use will expand in the future.

Some commenters were confused about how General permits work. A General permit will be issued only after the permitting authority has applied the Guidelines to the class of discharges to be covered by the permit. Therefore, there is no need to repeat the process at the time a particular discharge covered by the permit takes place. Of course, under both the Corps' regulations and EPA's regulations for State programs, the permitting authority may suspend General permits or require individual permits where environmental concerns make it appropriate. For example, cumulative impacts may turn out to be more serious than predicted. This regulation is not intended to establish the procedures for issuance of General permits. That is the responsibility of the permitting authority in accordance with the requirements of section 404.

Burden of Proof

A number of commenters objected to the presumption in the regulations in general, and in proposed Sec. 230.1(c) in particular, that dredged or fill material should not be discharged unless it is demonstrated that the planned discharge meets the Guidelines. These commenters thought that it was unfair and inconsistent with section 404(c) of the Act.

We disagree with these objections, and have retained the presumption against discharge and the existing burden of proof. However, the section has been rewritten for clarity.

The Clean Water Act itself declares a national goal to be the elimination of the discharge of pollutants into the navigable waters (section 101(a)(1)). This goal is implemented by section 301, which states that such discharges are unlawful except in compliance with, inter alia, section 404. Section 404 in turn authorizes the permitting authority to allow discharges of dredged or fill material if they comply with the 404(b)(1) Guidelines. The statutory scheme makes it clear that discharges shall not take place until they have been found acceptable. Of course, this finding may be made through the General permit process and the statutory exemptions as well as through individual permits.

The commenters who argued that section 404(c) shifts the usual burden to the EPA Administrator misunderstood the relationship between section 404(c) and the permitting process. The Administrator's authority to prohibit or restrict a site under section 404(c) operates independently of the Secretary of the Army's permitting authority in 404(a). The Administrator may use 404(c) whether or not a permit application is pending. Conversely, the Secretary may deny a permit on the basis of the Guidelines, whether or not EPA initiates a 404(c) proceeding. If the Administrator uses his 404(c) "veto," then he does have the burden to justify his action, but that burden does not come into play until he begins a 404(c) proceeding (See 40 CFR Part 231).

Toxic Pollutants

Many commenters objected strenuously to the presumptions in the Guidelines that toxic pollutants on the section 307(a)(1) list are present in the aquatic environment unless demonstrated not to be, and that such pollutants are biologically available unless demonstrated otherwise. These commenters argued that rebutting these presumptions could involve individual testing for dozens of substances every time a discharge is proposed, imposing an onerous task.

The proposed regulation attempted to avoid unnecessary testing by providing that when the Sec. 230.22(b) "reason to believe" process indicated that toxics were not present in the discharge material, no testing was required. On the other hand, contaminants other than toxics required testing if that same "reason to believe" process indicated they might be present in the discharge material. This is in fact a distinction without a difference. In practical application, toxic and non-toxic contaminants are treated the same; if either may be there, tests are performed to get the information for the determinations; if it is believed they are not present, no testing is done. Because the additional presumption for toxics did not actually serve a purpose, and because it was a possible source of confusion, we have eliminated it, and now treat "toxics" and other contaminants alike, under the "reason to believe test" (Sec. 230.60). We have provided in Sec. 230.3 a definition of "contaminants" which encompasses the 307(a)(1) toxics.

Water Dependency

One of the provisions in the proposed Guidelines which received the most objections was the so-called "water dependency test" in the proposed Sec. 230.10(e). This provision imposed an additional requirement on fills in wetlands associated with non-water dependent activities, namely a showing that the activity was "necessary." Many environmentalists objected to what they saw as a substantial weakening of the 1975 version of the water dependency test. Industry and development-oriented groups, on the other hand, objected to the "necessary" requirement because it was too subjective, and to the provision as a whole to the extent that it seemed designed to block discharges in wetlands automatically.

We have reviewed the water dependency test, its original purpose, and its relationship to the rest of the Guidelines in light of these comments. The original purpose, which many commenters commended, was to recognize the special values of wetlands and to avoid their unnecessary destruction, particularly when practicable alternatives were available in non-aquatic areas to achieve the basic purposes of the proposal. We still support this goal, but we have changed the water-dependency test to better achieve it.

First, we agree with the comments from both sides that the "necessary" test imposed by the 1979 proposal is not likely to be workable in practice, and may spawn more disputes than it settles. However, if the "necessary" test is simply deleted, section 230.10(e) does not provide any special recognition of or protection for wetlands, and thus defeats its purpose. Furthermore, even if the

"necessary" test were retained, the provision applies only to discharges of fill material, not discharges of dredged material, a distinction which lessens the effectiveness of the provision. Thus, we have decided, in accordance with the comments, that the proposal is unsatisfactory.

We have therefore decided to focus on, round out, and strengthen the approach of the so-called "water dependency" provision of the 1975 regulation. We have rejected the suggestion that we simply go back to the 1975 language, in part because it would not mesh easily with the revised general provisions of the Guidelines. Instead, our revised "water dependency" provision creates a presumption that there are practicable alternatives to "non-water dependent" discharges proposed for special aquatic sites. "Non-water dependent" discharges are those associated with activities which do not require access or proximity to or siting within the special aquatic site to fulfill their basic purpose. An example is a fill to create a restaurant site, since restaurants do not need to be in wetlands to fulfill their basic purpose of feeding people. In the case of such activities, it is reasonable to assume there will generally be a practicable site available upland or in a less vulnerable part of the aquatic ecosystem. The mere fact that an alternative may cost somewhat more does not necessarily mean it is not practicable (see Sec. 230.10(a)(2) and discussion below). Because the applicant may rebut the presumption through a clear showing in a given case, no unreasonable hardship should be worked. At the same time, this presumption should have the effect of forcing a hard look at the feasibility of using environmentally preferable sites. This presumption responds to the overwhelming number of commenters who urged us to retain a water dependency test to discourage avoidable discharges in wetlands.

In addition, the 1975 provision effectively created a special, irrebuttable presumption that alternatives to wetlands were always less damaging to the aquatic ecosystem. Because our experience and the comments indicate that this is not always the case, and because there could be substantial impacts on other elements of the environment and only minor impacts on wetlands, we have chosen instead to impose an explicit, but rebuttable, presumption that alternatives to discharges in special aquatic sites are less damaging to the aquatic ecosystem and are environmentally preferable. Of course, the general requirement that impacts on the aquatic ecosystem not be unacceptable also applies. The legislative history of the Clean Water Act, Executive Order 11990, and a large body of scientific information support this presumption.

Apart from the fact that it may be rebutted, this second presumption reincorporates the key elements of the 1975 provision. Moreover, it strengthens it because the recognition of the special environmental role of wetlands now applies to all discharges in special aquatic sites, whether of dredged or fill material, and whether or not water dependent. At the same time, this presumption, like the first one described above, retains sufficient flexibility to reflect the circumstances of unusual cases.

Consistent with the general burden of proof under these Guidelines, where an applicant proposes to discharge in a special aquatic site it is his responsibility to persuade the permitting authority that both of these presumptions have clearly been rebutted in order to pass the alternatives portion of these Guidelines.

Therefore, we believe that the new Sec. 230.10(a)(3), which replaces proposed 230.10(e), will give special protection to wetlands and other special aquatic sites regardless of material discharged, allay industry's concerns about the "necessary" test, recognize the possibility of impacts on air and upland systems, and acknowledge the variability among aquatic sites and discharge activities.

Alternatives

Some commenters objected at length to the scope of alternatives which the Guidelines require to be considered, and to the requirement that a permit be denied unless the least harmful such alternative were selected. Others wrote to urge us to retain these requirements. In our judgment, a number of the objections were based on a misunderstanding of what the proposed alternatives analysis required. Therefore, we have decided to clarify the regulation, but have not changed its basic thrust.

Section 403(c) clearly requires that alternatives be considered, and provides the basic legal basis for our requirement. While the statutory provision leaves the Agency some discretion to decide how alternatives are to be considered, we believe that the policies and goals of the Act, as well as the other authorities cited in the Preamble to the proposed Guidelines, would be best served by the approach we have taken.

First, we emphasize that the only alternatives which must be considered are practicable alternatives. What is practicable depends on cost, technical, and logistic factors. We have changed the word "economic" to "cost". Our intent is to consider those alternatives which are reasonable in terms of the overall scope/cost of the proposed project. The term economic might be construed to include consideration of the applicant's financial standing, or investment, or market share, a cumbersome inquiry which is not necessarily material to the objectives of the Guidelines. We consider it implicit that, to be practicable, an alternative must be capable of achieving the basic purpose of the proposed activity. Nonetheless, we have made this explicit to allay widespread concern. Both "internal" and "external" alternatives, as described in the September 18, 1979 Preamble, must satisfy the practicable test. In order for an "external" alternative to be practicable, it must be reasonably available or obtainable. However, the mere fact of ownership or lack thereof, does not necessarily determine reasonable availability. Some readers were apparently confused by the Preamble to the Proposed Regulation, which referred to the fact the National Environmental Policy Act (NEPA) may require consideration of courses of action beyond the authority of the agency involved. We did not mean to suggest that the Guidelines were necessarily imposing such a requirement on private individuals but, rather, to suggest that what we were requiring was well within the alternatives analyses required by NEPA.

Second, once these practicable alternatives have been identified in this fashion, the permitting authority should consider whether any of them, including land disposal options, are less environmentally harmful than the proposed discharge project. Of course, where there is no significant or easily identifiable difference in impact, the alternative need not be considered to have "less adverse" impact.

Several commenters questioned the legal basis for requiring the permitting authority to select the least damaging alternative. (The use of the term "select" may have been misleading. Strictly speaking, the permitting authority does not select anything; he denies the permit if the guidelines requirements have not been complied with.) As mentioned above, the statute leaves to EPA's discretion the exact implementation of the alternative requirement in section 403 of the Act. In large part, the approach taken by these regulations is very similar to that taken by the recent section 403(c) regulations (45 FR 65942, October 3, 1980). There is one difference; the Guidelines always prohibit discharges where there is a practicable, less damaging alternative, while the section 403(c) regulations only apply this prohibition in some cases. This difference reflects the wide range of water systems subject to 404 and the extreme sensitivity of many of them to physical destruction. These waters form a priceless mosaic. Thus, if destruction of an area of waters of the United States may reasonably be avoided, it should be avoided. Of course, where a category of 404 discharges is so minimal in its effects that it has been placed under a general permit, there is no need to perform a case-by-case alternatives analysis. This

feature corresponds, in a sense, to the category of discharges under section 403 for which no alternatives analysis is required.

Third, some commenters were concerned that the alternative consideration was unduly focused on water quality, and that a better alternative from a water quality standpoint might be less desirable from, say, an air quality point of view. This concern overlooks the explicit provision that the existence of an alternative which is less damaging to the aquatic ecosystem does not disqualify a discharge if that alternative has other significant adverse environmental consequences. This last provision gives the permitting authority an opportunity to take into account evidence of damage to other ecosystems in deciding whether there is a "better" alternative.

Fourth, a number of commenters were concerned that the Guidelines ensure coordination with planning processes under the Coastal Zone Management Act, Sec. 208 of the CWA, and other programs. We agree that where an adequate alternatives analysis has already been developed, it would be wasteful not to incorporate it into the 404 process. New Sec. 230.10(a)(5) makes it clear that where alternatives have been reviewed under another process, the permitting authority shall consider such analysis. However, if the prior analysis is not as complete as the alternatives analysis required under the Guidelines, he must supplement it as needed to determine whether the proposed discharge complies with the Guidelines. Section 230.10(a)(4) recognizes that the range of alternatives considered in NEPA documents will be sufficient for section 404 purposes, where the Corps is the permitting authority. (However, a greater level of detail may be needed in particular cases to be adequate for the 404(b)(1) Guidelines analysis.) This distinction between the Corps and State permitting authorities is based on the fact that it is the Corps' policy, in carrying out its own NEPA responsibilities, to supplement (or require a supplement to) a lead agency's environmental assessment or impact statement where such document does not contain sufficient information. State permitting agencies, on the other hand, are not subject to NEPA in this manner.

We have moved proposed Sec. 230.10(a)(1) (iii), concerning "other particular volumes and concentrations of pollutants at other specific rates", from the list of alternatives in Sec. 230.10 to Subpart H, Minimizing Adverse Effects, because it more properly belongs there.

Definitions (Sec. 230.3)

A number of the terms defined in Sec. 230.3 are also defined in the Corps' regulations at 33 CFR 323.2, applicable to the Corps' regulatory program. The Corps has recently proposed some revisions to those regulations and expects to receive comments on the definitions. To ensure coordination of these two sets of regulations, we have decided to reserve the definitions of "discharge of dredged material," "discharge of fill material," "dredged material," and "fill material," which otherwise would have appeared at Sec. 230.3 (f), (g), (j), and (l).

Although the term "waters of the United States" also appears in the Corps' regulations, we have retained a definition here, in view of the importance of this key jurisdictional term and the numerous comments received. The definition and the comments are explained below.

Until new definitions are published, directly or by reference to the Corps' revised regulations, users of these Guidelines should refer to the definitions in 33 CFR 323.2 (except in the case of state 404 programs, to which the definitions in 40 CFR Sec. 122.3 apply.)

Waters of the United States: A number of commenters objected to the definition of "waters of the United States" because it was allegedly outside the scope of the Clean Water Act or of the Constitution or because it was not identical to the Corps' definition. We have retained the proposed

definition with a few minor changes for clarity for several reasons. First, a number of courts have held that this basic definition of waters of the United States reasonably implements section 502(7) of the Clean Water Act, and that it is constitutional (e.g., United States v. Byrd, 609 F.2d 1204, 7th Cir. 1979; Leslie Salt Company v. Froehlke, 578 F.2d 742, 9th Cir. 1978). Second, we agree that it is preferable to have a uniform definition for waters of the United States, and for all regulations and programs under the CWA. We have decided to use the wording in the recent Consolidated Permit Regulations, 45 Fed. Reg. 33290, May 19, 1980, as the standard.*

Some commenters suggested that the reference in the definition to waters from which fish are taken to be sold in interstate commerce be expanded to include areas where such fish spawn. While we have not made this change because we wish to maintain consistency with the wording of the Consolidated Permit regulations, we do not intend to suggest that a spawning area may not have significance for commerce. The portion of the definition at issue lists major examples, not all the ways which commerce may be involved.

Some reviewers questioned the statement in proposed Sec. 230.72(c) (now Sec. 230.11(h)) that activities on fast land created by a discharge of dredged or fill material are considered to be in waters of the United States for purposes of these Guidelines. The proposed language was misleading and we have changed it to more accurately reflect our intent. When a portion of the Waters of the United States has been legally converted to fast land by a discharge of dredged or fill material, it does not remain waters of the United States subject to section 301(a). The discharge may be legal because it was authorized by a permit or because it was made before there was a permit requirement. In the case of an illegal discharge, the fast land may remain subject to the jurisdiction of the Act until the government determines not to seek restoration. However, in authorizing a discharge which will create fast lands, the permitting authority should consider, in addition to the direct effects of the fill inself, the effects on the aquatic environment of any reasonably foreseeable activities to be conducted on that fast land.

Section 230.54 (proposed 230.41) deals with impacts on parks, national and historical monuments, national sea shores, wilderness areas, research sites, and similar preserves. Some readers were concerned that we intended the Guidelines to apply to activities in such preserves whether or not the activities took place in waters of the United States. We intended, and we think the context makes it clear, that the Guidelines apply only to the specification of discharge sites in the waters of the United States, as defined in Sec. 230.3. We have included this section because the fact that a water of the United States may be located in one of these preserves is significant in evaluating the impacts of a discharge into that water.

Wetlands: Many wetlands are waters of the United States under the Clean Water Act. Wetlands are also the subject of Federal Executive Order No. 11990, and various Federal and State laws and regulations. A number of these other programs and laws have developed slightly different wetlands definitions, in part to accommodate or emphasize specialized needs. Some of these definitions include, not only wetlands as these Guidelines define them, but also mud flats and vegetated and unvegetated shallows. Under the Guidelines some of these other areas are grouped with wetlands as "Special Aquatic Sites" (Subpart E) and as such their values are given special recognition. (See discussion of Water Dependency above.) We agree with the comment that the National Inventory of Wetlands prepared by the U.S. Fish and Wildlife Service, while not necessarily exactly coinciding with the

*The Consolidated Permit Regulations exclude certain waste treatment systems from waters of the United States. The exact terms of this exclusion are undergoing technical revisions and are expected to change shortly. For this reason, these Guidelines as published do not contain the exclusion as originally worded in the Consolidated Permit Regulations. When published, the corrected exclusion will apply to the Guidelines as well as the Consolidated Permit Regulations.

scope of waters of the United States under the Clean Water Act or wetlands under these regulations, may help avoid construction in wetlands, and be a useful long-term planning tool.

Various commenters objected to the definition of wetlands in the Guidelines as too broad or too vague. This proposed definition has been upheld by the courts as reasonable and consistent with the Clean Water Act, and is being retained in the final regulation. However, we do agree that vegetative guides and other background material may be helpful in applying the definition in the field. EPA and the Corps are pledged to work on joint research to aid in jurisdictional determinations. As we develop such materials, we will make them available to the public.

Other commenters suggested that we expand the list of examples in the second sentence of the wetland definition. While their suggested additions could legally be added, we have not done so. The list is one of examples only, and does not serve as a limitation on the basic definition. We are reluctant to start expanding the list, since there are many kinds of wetlands which could be included, and the list could become very unwieldy.

In addition, we wish to avoid the confusion which could result from listing as examples, not only areas which generally fit the wetland definitions, but also areas which may or not meet the definition depending on the particular circumstances of a given site. In sum, if an area meets the definition, it is a wetland for purposes of the Clean Water Act, whether or not it falls into one of the listed examples. Of course, more often than not, it will be one of the listed examples.

A few commenters cited alleged inconsistencies between the definition of wetlands in Sec. 230.3 and Sec. 230.42. While we see no inconsistency, we have shortened the latter section as part of our effort to eliminate unnecessary comments.

Unvegetated Shallows: One of the special aquatic areas listed in the proposal was "unvegetated shallows" (Sec. 230.44). Since special aquatic areas are subject to the presumptions in Sec. 230.10(a)(3), it is important that they be clearly defined so that the permitting authority may readily know when to apply the presumptions. We were unable to develop, at this time, a definition for unvegetated shallows which was both easy to apply and not too inclusive or exclusive. Therefore, we have decided the wiser course is to delete unvegetated shallows from the special aquatic area classification. Of course, as waters of the United States, they are still subject to the rest of the Guidelines.

"Fill Material": We are temporarily reserving Sec. 230.3(1). Both the proposed Guidelines and the proposed Consolidated Permit Regulations defined fill material as material discharged for the primary purpose of replacing an aquatic area with dryland or of changing the bottom elevation of a water body, reserving to the NPDES program discharges with the same effect which are primarily for the purpose of disposing of waste. Both proposals solicited comments on this distinction, referred to as the primary purpose test. On May 19, 1980, acting under a court-imposed deadline, EPA issued final Consolidated Permit Regulations while the 404(b)(1) Guidelines rulemaking was still pending. These Consolidated Permit Regulations contained a new definition of fill material which eliminated the primary purpose test and included as fill material all pollutants which have the effect of fill, that is, which replace part of the waters of the United States with dryland or which change the bottom elevation of a water body for any purpose. This new definition is similar to the one used before 1977.

During the section 404(b)(1) rulemaking, the Corps has raised certain questions about the implementation of such a definition. Because of the importance of making the Final Guidelines available without further delay, and because of our desire to cooperate with the Corps in resolving their concerns about fill material, we have decided to temporarily reserve Sec. 230.3(1) pending further

discussion. This action does not affect the effectiveness of the Consolidated Permit Regulations. Consequently, there is a discrepency between those regulations and the Corps' regulations, which still contain the old definition.

Therefore, to avoid any uncertainty from this situation, EPA wishes to make clear its enforcement policy for unpermitted discharges of solid waste. EPA has authority under section 309 of the CWA to issue administrative orders against violations of section 301. Unpermitted discharges of solid waste into waters of the United States violate section 301.

Under the present circumstances, EPA plans to issue solid waste administrative orders with two basic elements. First, the orders will require the violator to apply to the Corps of Engineers for a section 404 permit within a specified period of time. (The Corps has agreed to accept these applications and to hold them until it resolves its position on the definition of fill material.)

Second, the order will constrain further discharges by the violator. In extreme cases, an order may require that discharges cease immediately. However, because we recognize that there will be a lapse of time before decisions are made on this kind of permit application, these orders may expressly allow unpermitted discharges to continue subject to specific conditions set forth by EPA in the order. These conditions will be designed to avoid further environmental damage.

Of course, these orders will not influence the ultimate issuance or non-issuance of a permit or determine the conditions that may be specified in such a permit. Nor will such orders limit the Administrator's authority under section 309(b) or the right of a citizen to bring suit against a violator under section 505 of the CWA.

Permitting Authority: We have used the new term "permitting authority," instead of "District Engineer," throughout these regulations, in recognition of the fact that under the 1977 amendments approved States may also issue permits.

Coastal Zone Management Plans

Several commenters were concerned about the relationship between section 404 and approved Coastal Zone Management (CZM) plans. Some expressed concern that the Guidelines might authorize a discharge prohibited by a CZM plan; others objected to the fact that the Guidelines might prohibit a discharge which was consistent with a CZM plan.

Under section 307(b) of the CZM Act, no Federal permits may be issued until the applicant furnishes a certification that the discharge is consistent with an approved CZM plan, if there is one, and the State concurs in the certification or waives review. Section 325.2(b)(2) of the Corps' regulation, which applies to all Federal 404 permits, implements this requirement for section 404. Because the Corps' regulations adequately address the CZM consistency requirement, we have not duplicated Sec. 325.2(b)(2) in the Guidelines. Where a State issues State 404 permits, it may of course require consistency with its CZM plan under State law.

The second concern, that the 404 Guidelines might be stricter than a CZM plan, points out a possible problem with CZM plans, not with the Guidelines. Under 307(f) of CZMA, all CZM plans must provide for compliance with applicable requirements of the Clean Water Act. The Guidelines are one such requirement. Of course, to the extent that a CZM plan is general and area-wide, it may be impossible to include in its development the same project-specific consideration of impacts and alternatives required under the Guidelines. Nonetheless, it cannot authorize or mandate a discharge of dredged or fill material which fails to comply with the requirements of these Guidelines. Often CZM

plans contain a requirement that all activities conducted under it meet the permit requirements of the Clean Water Act. In such a case, there could of course be no conflict between the CZM plan and the requirements of the Guidelines.

We agree with commenters who urge that delay and duplication of effort be avoided by consolidating alternatives studies required under different statutes, including the Coastal Zone Management Act. However, since some planning processes do not deal with specific projects, their consideration of alternatives may not be sufficient for the Guidelines. Where another alternative analysis is less complete than that contemplated under section 404, it may not be used to weaken the requirements of the Guidelines.

Advanced Identification of Dredged or Fill Material Disposal Sites

A large number of commenters objected to the way proposed Sec. 230.70, new Subpart I, had been changed from the 1975 regulations. A few objected to the section itself. Most of the comments also revealed a misunderstanding about the significance of identifying an area. First, the fact that an area has been identified as unsuitable for a potential discharge site does not mean that someone cannot apply for and obtain a permit to discharge there as long as the Guidelines and other applicable requirements are satisified.* Conversely, the fact that an area has been identified as a potential site does not mean that a permit is unnecessary or that one will automatically be forthcoming. The intent of this section was to aid applicants by giving advance notice that they would have a relatively easy or difficult time qualifying for a permit to use particular areas. Such advance notice should facilitate applicant planning and shorten permit processing time.

Most of the objectors focused on EPA's "abandonment" of its "authority" to identify sites. While that "authority" is perhaps less "authoritative" than the commenters suggested (see above), we agree that there is no reason to decrease EPA's role in the process. Therefore, we have changed new Sec. 230.80(a) to read:

"Consistent with these Guidelines, EPA and the permitting authority on their own initiative or at the request of any other party, and after consultation with any affected State that is not the permitting authority, may identify sites which will be considered as:"

We have also deleted proposed Sec. 230.70(a)(3), because it did not seem to accomplish much. Consideration of the point at which cumulative and secondary impacts become unacceptable and warrant emergency action will generally be more appropriate in a permit-by-permit context. Once that point has been so determined, of course, the area can be identified as "unsuitable" under the new Sec. 230.80(a)(2).

Executive Order 12044

A number of commenters took the position that Executive Order 12044 requires EPA to prepare a "regulatory analysis" in connection with these regulations. EPA disagrees. These regulations are not, strictly speaking, new regulations. They do not impose new standards or requirements, but rather substantially clarify and reorganize the existing interim final regulations

*EPA may foreclose the use of a site by exercising its authority under section 404(c). The advance identification referred to in this section is not a section 404(c) prohibition.

Under EPA's criteria implementing Executive Order 12044, EPA will prepare a Regulatory Analysis for any regulation which imposes additional annual costs totalling $100 million or which will result in a total additional cost of production of any major product or service which exceeds 5% of its selling price. While many commenters, particularly members of the American Association of Port Authorities (AAPA), requested a regulatory analysis and claimed that the regulations were too burdensome, none of them explained how that burden was an additional one attributable to this revision. A close comparison of the new regulation and the explicit and implicit requirements in the interim final Guidelines reveals that there has been very little real change in the criteria by which discharges are to be judged or in the tests that must be conducted; therefore, we stand by our original determination that a regulatory analysis is not required.

Perhaps the most significant area in which the regulations are more explicit and arguably stricter is in the consideration of alternatives. However, even the 1975 regulations required the permitting authority to consider "the availability of alternate sites and methods of disposal that are less damaging to the environment," and to avoid activities which would have significant adverse effects. We do not think that the revised Guidelines' more explicit direction to avoid adverse effects that could be prevented through selection of a clearly less damaging site or method is a change imposing a substantial new burden on the regulated public.

Because the revised regulations are more explicit than the interim final regulations in some respects, it is possible that permit reviewers will do a more thorough job evaluating proposed discharges. This may result in somewhat more carefully drawn permit conditions. However, even if, for purposes of argument, the possible cost of complying with these conditions is considered an additional cost, there is no reason to believe that it alone will be anywhere near $100 million annually.

We also believe that it is appropriate to recognize the regulatory benefits from these more carefully drafted final regulations. Because they are much clearer about what should be considered and documented, we expect there will be fewer delays in reviewing permits, and that initial decisions to issue permits are less likely to be appealed to higher authority. These benefits are expected to offset any potential cost increase.

Some commenters suggested that documentation requirements would generate an additional cost of operations. The Corps' procedural regulations at 33 CFR 325.8 and 325.11 already require extensive documentation for individual permits being denied or being referred to higher authority for resolution of a conflict between agencies.

Economic Factors

A number of commenters asked EPA to include consideration of economic factors in the Guidelines. We believe that the regulation already recognizes economic factors to the extent contemplated by the statute. First, the Guidelines explicitly include the concept of "practicability" in connection with both alternatives and steps to minimize impacts. If an alleged alternative is unreasonably expensive to the applicant, the alternative is not "practicable." In addition, the Guidelines also consider economics indirectly in that they are structured to avoid the expense of unnecessary testing through the "reason-to-believe-test." Second, the statute expressly provides that the economics of anchorage and navigation may be considered, but only after application of the section 404(b)(1) Guidelines. (See section 404(b)(2).)

Borrow Sites

A number of highway departments objected because they felt the Guidelines would require them to identify specific borrow sites at the time of application, which would disrupt their normal contracting process and increase cost. These objections were based on a misunderstanding of the Guideline's requirements. Under those Guidelines, the actual borrow sites need not be identified, if the application and the permit specify that the discharge material must come from clean upland sites which are removed from sources of contamination and otherwise satisfy the reason-to-believe test. A condition that the material come from such a site would enable the permitting authority to make his determinations and find compliance with the conditions of Sec. 230.10, without requiring highway departments to specify in advance the specific borrow sites to be used.

Consultation With Fish and Wildlife Agencies

One commenter wanted us to put in a statement that the Fish and Wildlife Coordination Act requires consultation with fish and wildlife agencies. We have not added new language because (1) the Fish and Wildlife Act only applies to Federal permitting agencies and not to State permitting agencies, and (2) the Corps' regulations already provide for such consultation by the only Federal 404 permitting agency. However, we agree with the commenter that Federal and State fish and wildlife agencies may often provide valuable assistance in evaluating the impacts of discharges of dredged or fill material.

The Importance of Appropriate Documentation

Specific documentation is important to ensure an understanding of the basis for each decision to allow, condition, or prohibit a discharge through application of the Guidelines. Documentation of information is required for: (1) facts and data gathered in the evaluation and testing of the extraction site, the material to be discharged, and the disposal site; (2) factual determinations regarding changes that can be expected at the disposal site if the discharge is made as proposed; and (3) findings regarding compliance with Sec. 230.10 conditions. This documentation provides a record of actions taken that can be evaluated for adequacy and accuracy and ensures consideration of all important impacts in the evaluation of a proposed discharge of dredged or fill material.

The specific information documented under (1) and (2) above in any given case depends on the level of investigation necessary to provide for a reasonable understanding of the impact on the aquatic ecosystems. We anticipate that a number of individual and most General permit applications will be for routine, minor activities with little potential for significant adverse environmental impacts. In such cases, the permitting authority will not have to require extensive testing or analysis to make his findings of compliance. The level of documentation should reflect the significance and complexity of the proposed discharge activity.

Factual Determinations

Proposed section 230.20, "Factual Determinations" (now Sec. 230.11) has been significantly reorganized in response to comments. First, we have changed (e) to reflect our elimination of the artificial distinction between the section 307(a)(1) toxics and other contaminants. Second, we have eliminated proposed (f) (Biological Availability), since the necessary information will be provided by (d) and new (e). Proposed (f) was intended to reflect the presumption that toxics were present and biologically available. We have modified proposed (g), now (f), to focus on the size of the disposal site and the size and shape of the mixing zone. The specific requirement to document the site has been deleted; where such information is relevant, it will automatically be considered in making the other determinations. We have also deleted proposed (h) (Special Determinations) since it did not provide

any useful information which would not already be considered in making the other factual determinations.

Finally, in response to many comments, we have moved the provisions on cumulative and secondary impact to the Factual Determination section to give them further emphasis. We agree that such impacts are an important consideration in evaluating the acceptability of a discharge site.

Water Quality Standards

One commenter was concerned that the reference Sec. 230.10(b) to water quality standards and criteria "approved or promulgated under section 303" might encourage permit authorities to ignore other water quality requirements. Under section 303, all State water quality standards are to be submitted to EPA for approval. If the submitted standards are incomplete or insufficiently stringent, EPA may promulgate standards to replace or supplant the State standards. Disapproved standards remain in effect until replaced. Therefore, to refer to "EPA approved or promulgated standards" is to ignore those State standards which have been neither approved nor replaced. We have therefore changed the wording of this requirement as follows: "* * * any applicable State water quality standard." We have also dropped the reference to "criteria", to be consistent with the Agency's general position that water quality criteria are not regulatory.

Other Requirements for Discharge

Section 230.10(c) provides that discharges are not permitted if they will have "significantly" adverse effects on various aquatic resources. In this context, "significant" and "significantly" mean more than "trivial", that is, significant in a conceptual rather than a statistical sense. Not all effects which are statistically significant in the laboratory are significantly adverse in the field.

Section 320.10(d) uses the term "minimize" to indicate that all reasonable reduction in impacts be obtained. As indicated by the "appropriate and practicable" provision, steps which would be unreasonably costly or would be infeasible or which would accomplish only inconsequential reductions in impact need not be taken.

Habitat Development and Restoration of Water Bodies

Habitat development and restoration involve changes in open water and wetlands that minimize adverse effects of proposed changes or that neutralize or reverse the effects of past changes on the ecosystem. Development may produce a new or modified ecological state by displacement of some or all of the existing environmental characteristics. Restoration has the potential to return degraded environments to their former ecological state.

Habitat development and restoration can contribute to the maintenance and enhancement of a viable aquatic ecosystem at the discharge site. From an environmental point of view, a project involving the discharge of dredged and fill material should be designed and managed to emulate a natural ecosystem. Research, demonstration projects, and full scale implementation have been done in many categories of development and restoration. The U.S. Fish and Wildlife Service has programs to develop and restore habitat. The U.S. Army Engineer Waterways Experiment Station has published guidelines for using dredged material to develop wetland habitat, for establishing marsh vegetation, and for building islands that attract colonies of nesting birds. The EPA has a Clean Lakes program which supplies funds to States and localities to enhance or restore degraded lakes. This may involve dredging nutrient-laden sediments from a lake and ensuring that nutrient inflows to the lake are controlled. Restoration and habitat development techniques can be used to minimize adverse impacts

and compensate for destroyed habitat. Restoration and habitat development may also provide secondary benefits such as improved opportunities for outdoor recreation and positive use for dredged materials.

The development and restoration of viable habitats in water bodies requires planning and construction practices that integrate the new or improved habitat into the existing environment. Planning requires a model or standard, the achievement of which is attempted by manipulating design and implementation of the activity. This model or standard should be based on characteristics of a natural ecosystem in the vicinity of a proposed activity. Such use of a natural ecosystem ensures that the developed or restored area, once established, will be nourished and maintained physically, chemically and biologically by natural processes. Some examples of natural ecosystems include, but are not limited to, the following: salt marsh, cattail marsh, turtle grass bed, small island, etc.

Habitat development and restoration, by definition, should have environmental enhancement and maintenance as their initial purpose. Human uses may benefit but they are not the primary purpose. Where such projects are not founded on the objectives of maintaining ecosystem function and integrity, some values may be favored at the expense of others. The ecosystem affected must be considered in order to achieve the desired result of development and restoration. In the final analysis, selection of the ecosystem to be emulated is of critical importance and a loss of value can occur if the wrong model or an incomplete model is selected. Of equal importance is the planning and management of habitat development and restoration on a case-by-case basis.

Specific measures to minimize impacts on the aquatic ecosystem by enhancement and restoration projects include but are not limited to:

(1) Selecting the nearest similar natural ecosystem as the model in the implementation of the activity.

Obviously degraded or significantly less productive habitats may be considered prime candidates for habitat restoration. One viable habitat, however, should not be sacrificed in an attempt to create another, i.e., a productive vegetated shallow water area should not be destroyed in an attempt to create a wetland in its place.

(2) Using development and restoration techniques that have been demonstrated to be effective in circumstances similar to those under consideration wherever possible.

(3) Where development and restoration techniques proposed for use have not yet advanced to the pilot demonstration or implementation stage, initiate their use on a small scale to allow corrective action if unanticipated adverse impacts occur.

(4) Where Federal funds are spent to clean up waters of the U.S. through dredging, scientifically defensible levels of pollutant concentration in the return discharge should be agreed upon with the funding authority in addition to any applicable water quality standards in order to maintain the desired improved water quality.

(5) When a significant ecological change in the aquatic environment is proposed by the discharge of dredged or fill material, the permitting authority should consider the ecosystem that will be lost as well as the environmental benefits of the new system.

Dated: December 12, 1980.

Douglas M. Costle,

Administrator, Environmental Protection Agency.

Part 230 is revised to read as follows:

PART 230 -- SECTION 404(b)(1) GUIDELINES FOR SPECIFICATION OR DISPOSAL SITES FOR DREDGED OF FILL MATERIAL

Subpart A -- General

Sec.

230.1 Purpose and policy.

230.2 Applicability.

230.3 Definitions.

230.4 Organization.

230.5 General procedures to be followed.

230.6 Adaptability.

230.7 General permits.

Subpart B -- Compliance With the Guidelines

230.10 Restrictions on discharge.

230.11 Factual determinations.

230.12 Findings of compliance or non-compliance with the restrictions on discharge.

Subpart C -- Potential Impacts on Physical and Chemical Characteristics of the Aquatic Ecosystem

230.20 Substrate.

230.21 Suspended particulates/turbidity.

230.22 Water.

230.23 Current patterns and water circulation.

230.24 Normal water fluctuations.

230.25 Salinity gradients.

Subpart D -- Potential Impacts on Biological Characteristics of the Aquatic Ecosystem

230.30 Threatened and endangered species.

230.31 Fish, crustaceans, mollusks, and other aquatic organisms in the food web.

230.32 Other wildlife.

Subpart E -- Potential Impacts on Special Aquatic Sites

230.40 Sanctuaries and refuges.

230.41 Wetlands.

230.42 Mud flats.

230.43 Vegetated shallows.

230.44 Coral reefs.

230.45 Riffle and pool complexes.

Subpart F -- Potential Effects on Human Use Characteristics

230.50 Municipal and private water supplies.

230.51 Recreational and commercial fisheries.

230.52 Water-related recreation.

230.53 Aesthetics.

230.54 Parks, national and historic monuments, national seashores, wilderness areas, research sites and similar preserves.

Subpart G -- Evaluation and Testing

230.60 General evaluation of dredged or fill material.

230.61 Chemical, biological, and physical evaluation and testing.

Subpart H -- Actions to Minimize Adverse Effects

230.70 Actions concerning the location of the discharge.

230.71 Actions concerning the material to be discharged.

230.72 Actions controlling the material after discharge.

230.73 Actions affecting the method of dispersion.

230.74 Actions related to technology.

230.75 Actions affecting plant and animal populations.

230.76 Actions affecting human use.

230.77 Other actions.

Subpart I -- Planning To Shorten Permit Processing Time

230.80 Advanced identification of disposal areas.

Authority: This regulation is issued under authority of Sections 404(b) and 501(a) of the Clean Water Act of 1977, 33 U.S.C. Sec. 1344(b) and Sec. 1361(a).

Subpart A -- General

Sec. 23.1 Purpose and policy.

(a) The purpose of these Guidelines is to restore and maintain the chemical, physical, and biological integrity of waters of the United States through the control of discharges of dredged or fill material.

(b) Congress has expressed a number of policies in the Clean Water Act. These Guidelines are intended to be consistent with and to implement those policies.

(c) Fundamental to these Guidelines is the precept that dredged or fill material should not be discharged into the aquatic ecosystem, unless it can be demonstrated that such a discharge will not have an unacceptable adverse impact either individually or in combination with known and/or probable impacts of other activities affecting the ecosystems of concern.

(d) From a national perspective, the degradation or destruction of special aquatic sites, such as filling operations in wetlands, is considered to be among the most severe environmental impacts covered by these Guidelines. The guiding principle should be that degradation or destruction of special sites may represent an irreversible loss of valuable aquatic resources.

Sec. 230.2 Applicability.

(a) These Guidelines have been developed by the Administrator of the Environmental Protection Agency in conjunction with the Secretary of the Army acting through the Chief of Engineers under section 404(b)(1) of the Clean Water Act (33 U.S.C. 1344). The Guidelines are applicable to the specification of disposal sites for discharges of dredged or fill material into waters of the United States. Sites may be specified through:

(1) The regulatory program of the U.S. Army Corps of Engineers under sections 404(a) and (e) of the Act (see 33 CFR 320, 323 and 325);

(2) The civil works program of the U.S. Army Corps of Engineers (see 33 CFR 209.145 and section 150 of Pub. L. 94-587, Water Resources Development Act of 1976);

(3) Permit programs of States approved by the Administrator of the Environmental Protection Agency in accordance with sections 404(g) and (h) of the Act (see 40 CFR 122, 123 and 124); (4) Statewide dredged or fill material regulatory programs with best management practices approved under section 208(b)(4)(B) and (C) of the Act (see 40 CFR 35.1560);

(5) Federal construction projects which meet criteria specified in section 404(r) of the Act.

(b) These Guidelines will be applied in the review of proposed discharges of dredged or fill material into navigable waters which lie inside the baseline from which the territorial sea is measured, and the discharge of fill material into the territorial sea, pursuant to the procedures referred to in paragraphs (a)(1) and (a)(2) above. The discharge of dredged material into the territorial sea is governed by the Marine Protection, Research, and Sanctuaries Act of 1972, Pub. L. 92-532, and regulations and criteria issued pursuant thereto (40 CFR Part 220-228).

(c) Guidance on interpreting and implementing these Guidelines may be prepared jointly by EPA and the Corps at the national or regional level from time to time. No modifications to the basic application, meaning, or intent of these Guidelines will be made without rulemaking by the Administrator under the Administrative Procedure Act (5 U.S.C. 551 et seq.).

Sec. 230.3 Definitions.

For purposes of this Part, the following terms shall have the meanings indicated:

(a) The term "Act" means the Clean Water Act (also known as the Federal Water Pollution Control Act or FWPCA) Pub. L. 92-500, as amended by Pub. L. 95-217, 33 U.S.C. 1251, et seq.

(b) The term "adjacent"means bordering, contiguous, or neighboring. Wetlands separated from other waters of the United States by man-made dikes or barriers, natural river berms, beach dunes, and the like are "adjacent wetlands."

(c) The terms "aquatic environment" and "aquatic ecosystem" mean waters of the United States, including wetlands, that serve as habitat for interrelated and interacting communities and populations of plants and animals.

(d) The term "carrier of contaminant" means dredged or fill material that contains contaminants.

(e) The term "contaminant" means a chemical or biological substance in a form that can be incorporated into, onto or be ingested by and that harms aquatic organisms, consumers of aquatic organisms, or users of the aquatic environment, and includes but is not limited to the substances on the 307(a)(1) list of toxic pollutants promulgated on January 31, 1978 (43 FR 4109).

(f) [Reserved]

(g) [Reserved]

(h) The term "discharge point" means the point within the disposal site at which the dredged or fill material is released.

(i) The term "disposal site" means that portion of the "waters of the United States" where specific disposal activities are permitted and consist of a bottom surface area and any overlying volume of water. In the case of wetlands on which surface water is not present, the disposal site consists of the wetland surface area.

(j) [Reserved]

(k) The term "extraction site" means the place from which the dredged or fill material proposed for discharge is to be removed.

(l) [Reserved]

(m) The term "mixing zone" means a limited volume of water serving as a zone of initial dilution in the immediate vicinity of a discharge point where receiving water quality may not meet quality standards or other requirements otherwise applicable to the receiving water. The mixing zone should be considered as a place where wastes and water mix and not as a place where effluents are treated.

(n) The term "permitting authority" means the District Engineer of the U.S. Army Corps of Engineers or such other individual as may be designated by the Secretary of the Army to issue or deny permits under section 404 of the Act; or the State Director of a permit program approved by EPA under Sec. 404(g) and Sec. 404(h) or his delegated representative.

(o) The term "pollutant" means dredged spoil, solid waste, incinerator residue, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials not covered by the Atomic Energy Act, heat, wrecked or discarded equipment, rock, sand, cellar dirt, and industrial, municipal, and agricultural waste discharged into water. The legislative history of the Act reflects that "radioactive materials" as included within the definition of "pollutant" in section 502 of the Act means only radioactive materials which are not encompassed in the definition of source, byproduct, or special nuclear materials as defined by the Atomic Energy Act of 1954, as amended, and regulated under the Atomic Energy Act. Examples of radioactive materials not covered by the Atomic Energy Act and, therefore, included within the term "pollutant", are radium and accelerator produced isotopes. See Train v. Colorado Public Interest Research Group, Inc., 426 U.S. 1 (1976).

(p) The term "pollution" means the man-made or man-induced alteration of the chemical, physical, biological or radiological integrity of an aquatic ecosystem.

(q) The term "practicable" means available and capable of being done after taking into consideration cost, existing technology, and logistics in light of overall project purposes.

(q-1) "Special aquatic sites" means those sites identified in Subpart E. They are geographic areas, large or small, possessing special ecological characteristics of productivity, habitat, wildlife protection, or other important and easily disrupted ecological values. These areas are generally recognized as significantly influencing or positively contributing to the general overall environmental health or vitality of the entire ecosystem of a region. (See 230.10(a)(3)) (r) The term "territorial sea" means the belt of the sea measured from the baseline as determined in accordance with the Conventon on the Territorial Sea and the Contiguous Zone and extending seaward a distance of three miles.

(s) The term "waters of the united States" means:

(1) All waters which are currently used, or were used in the past, or may be susceptible to use in interstate or foreign commerce, including all waters which are subject to the ebb and flow of the tide;

(2) All interstate waters including interstate wetlands;

(3) All other waters such as intrastate lakes, rivers, streams (including intermittent streams), mudflats, sandflats, wetlands, sloughs, prairie potholes, wet meadows, playa lakes, or natural ponds, the use, degradation or destruction of which could affect interstate or foreign commerce including any such waters:

(i) Which are or could be used by interstate or foreign travelers for recreational or other purposes; or

(ii) From which fish or shellfish are or could be taken and sold in interstate or foreign commerce; or

(iii) Which are used or could be used for industrial purposes by industries in interstate commerce;

(4) All impoundments of waters otherwise defined as waters of the United States under this definition.

(5) Tributaries of waters identified in paragraphs (1)-(4) of this section;

(6) The territorial sea;

(7) Wetlands adjacent to waters (other than waters that are themselves wetlands) identified in paragraphs (s) (1)-(6) of this section; waste treatment systems, including treatment ponds or lagoons designed to meet the requirements of CWA (other than cooling ponds as defined in 40 CFR Sec. 423.11(m) which also meet the criteria of this definition) are not waters of the United States.

(t) The term "wetlands" means those areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs and similar areas.

Sec. 230.4 Organization.

The Guidelines are divided into eight subparts. Subpart A presents those provisions of general applicability, such as purpose and definitions. Subpart B establishes the four conditions which must be satisfied in order to make a finding that a proposed discharge of dredged or fill material complies with the Guidelines. Section 230.11 of Subpart B, sets forth factual determinations which are to be considered in determining whether or not a proposed discharge satisfies the Subpart B conditions of compliance. Subpart C describes the physical and chemical components of a site and provides guidance as to how proposed discharges of dredged or fill material may affect these components. Subparts D-F detail the special characteristics of particular aquatic ecosystems in terms of their values, and the possible loss of these values due to discharges of dredged or fill material. Subpart G prescribes a number of physical, chemical, and biological evaluations and testing procedures to be used in reaching the required factual determinations. Subpart H details the means to prevent or mimimize adverse effects. Subpart I concerns advanced identification of disposal areas.

Sec. 230.5 General procedures to be followed.

In evaluating whether a particular discharge site may be specified, the permitting authority should use these Guidelines in the following sequence:

(a) In order to obtain an overview of the principal regulatory provisions of the Guidelines, review the restrictions on discharge in Sec. 230.10(a)-(d), the measures to mimimize adverse impact of Subpart H, and the required factual determinations of Sec. 230.11.

(b) Determine if a General permit (Sec. 230.7) is applicable; if so, the applicant needs merely to comply with its terms, and no further action by the permitting authority is necessary. Special conditions for evaluation of proposed General permits are contained in Sec. 230.7. If the discharge is not covered by a General permit:

(c) Examine practicable alternatives to the proposed discharge, that is, not discharging into the waters of the U.S. or discharging into an alternative aquatic site with potentially less damaging consequences (Sec. 230.10(a)).

(d) Delineate the candidate disposal site consistent with the criteria and evaluations of Sec. 230.11(f).

(e) Evaluate the various physical and chemical components which characterize the non-living environment of the candidate site, the substrate and the water including its dynamic characteristics (Subpart C).

(f) Identify and evaluate any special or critical characteristics of the candidate disposal site, and surrounding areas which might be affected by use of such site, related to their living communities or human uses (Subparts D, E, and F).

(g) Review Factual Determinations in Sec. 230.11 to determine whether the information in the project file is sufficient to provide the documentation required by Sec. 230.11 or to perform the pre-testing evaluation described in Sec. 230.60, or other information is necessary.

(h) Evaluate the material to be discharged to determine the possibility of chemical contamination or physical incompatibility of the material to be discharged (Sec. 230.60).

(i) If there is a reasonable probability of chemical contamination, conduct the appropriate tests according to the section on Evaluation and Testing (Sec. 230.61).

(j) Identify appropriate and practicable changes to the project plan to minimize the environmental impact of the discharge, based upon the specialized methods of minimization of impacts in Subpart H.

(k) Make and document Factual Determinations in Sec. 230.11.

(l) Make and document Findings of Compliance (Sec. 230.12) by comparing Factual Determinations with the requirements for discharge of Sec. 230.10.

This outline of the steps to follow in using the Guidelines is simplified for purposes of illustration. The actual process followed may be iterative, with the results of one step leading to a reexamination of previous steps. The permitting authority must address all of the relevant provisions of the Guidelines in reaching a Finding of Compliance in an individual case.

Sec. 230.6 Adaptability.

(a) The manner in which these Guidelines are used depends on the physical, biological, and chemical nature of the proposed extraction site, the material to be discharged, and the candidate disposal site, including any other important components of the ecosystem being evaluated. Documentation to demonstrate knowledge about the extraction site, materials to be extracted, and the candidate disposal site is an essential component of guideline application. These Guidelines allow evaluation and documentation for a variety of activities, ranging from those with large, complex impacts on the aquatic environment to those for which the impact is likely to be innocuous. It is unlikely that the Guidelines will apply in their entirety to any one activity, no matter how complex. It is anticipated that substantial numbers of permit applications will be for minor, routine activities that have little, if any, potential for significant degradation of the aquatic environment. It generally is not intended or expected that extensive testing, evaluation or analysis will be needed to make findings of compliance

in such routine cases. Where the conditions for General permits are met, and where numerous applications for similar activities are likely, the use of General permits will eliminate repetitive evaluation and documentation for individual discharges.

(b) The Guidelines user, including the agency or agencies responsible for implementing the Guidelines, must recognize the different levels of effort that should be associated with varying degrees of impact and require or prepare commensurate documentation. The level of documentation should reflect the significance and complexity of the discharge activity.

(c) An essential part of the evaluation process involves making determinations as to the relevance of any portion(s) of the Guidelines and conducting further evaluation only as needed. However, where portions of the Guidelines review procedure are "short form" evaluations, there still must be sufficient information (including consideration of both individual and cumulative impacts) to support the decision of whether to specify the site for disposal of dredged or fill material and to support the decision to curtail or abbreviate the evaluation process. The presumption against the discharge in Sec. 230.1 applies to this decision-making.

(d) In the case of activities covered by General permits or 208(b)(4)(B) and (C) Best Management Practices, the analysis and documentation required by the Guidelines will be performed at the time of General permit issuance or 208(b)(4)(B) and (C) Best Management Practices promulgation and will not be repeated when activities are conducted under a General permit or 208(b)(4)(B) and (C) Best Management Practices control. These Guidelines do not require reporting or formal written communication at the time individual activities are initiated under a General permit or 208(b)(4)(B) and (C) Best Management Practices. However, a particular General permit may require appropriate reporting.

Sec. 230.7 General permits.

(a) Conditions for the issuance of General permits. A General permit for a category of activities involving the discharge of dredged or fill material complies with the Guidelines if it meets the applicable restrictions on the discharge in Sec. 230.10 and if the permitting authority determines that:

(1) The activities in such category are similar in nature and similar in their impact upon water quality and the aquatic environment;

(2) The activities in such category will have only minimal adverse effects when performed separately; and

(3) The activities in such category will have only minimal cumulative adverse effects on water quality and the aquatic environment.

(b) Evaluation process. To reach the determinations required in paragraph (a) of this section, the permitting authority shall set forth in writing an evaluation of the potential individual and cumulative impacts of the category of activities to be regulated under the General permit. While some of the information necessary for this evaluation can be obtained from potential permittees and others through the proposal of General permits for public review, the evaluation must be completed before any General permit is issued, and the results must be published with the final permit.

(1) This evaluation shall be based upon consideration of the prohibitions listed in Sec. 230.10(b) and the factors listed in Sec. 230.10(c), and shall include documented information supporting each factual

determination in Sec. 230.11 of the Guidelines (consideration of alternatives in Sec. 230.10(a) are not directly applicable to General permits);

(2) The evaluation shall include a precise description of the activities to be permitted under the General permit, explaining why they are sufficiently similar in nature and in environmental impact to warrant regulation under a single General permit based on Subparts C-F of the Guidelines. Allowable differences between activities which will be regulated under the same General permit shall be specified. Activities otherwise similar in nature may differ in environmental impact due to their location in or near ecologically sensitive areas, areas with unique chemical or physical characteristics, areas containing concentrations of toxic substances, or areas regulated for specific human uses or by specific land or water management plans (e.g., areas regulated under an approved Coastal Zone Management Plan). If there are specific geographic areas within the purview of a proposed General permit (called a draft General permit under a State 404 program), which are more appropriately regulated by individual permit due to the considerations cited in this paragraph, they shall be clearly delineated in the evaluation and excluded from the permit. In addition, the permitting authority may require an individual permit for any proposed activity under a General permit where the nature or location of the activity makes an individual permit more appropriate.

(3) To predict cumulative effects, the evaluation shall include the number of individual discharge activities likely to be regulated under a General permit until its expiration, including repetitions of individual discharge activities at a single location.

Subpart B -- Compliance With the Guidelines

Sec. 230.10 Restrictions on discharge.

Note. -- Because other laws may apply to particular discharges and because the Corps of Engineers or State 404 agency may have additional procedural and substantive requirements, a discharge complying with the requirement of these Guidelines will not automatically receive a permit.

Although all requirements in Sec. 230.10 must be met, the compliance evaluation procedures will vary to reflect the seriousness of the potential for adverse impacts on the aquatic ecosystems posed by specific dredged or fill material discharge activities.

(a) Except as provided under Sec. 404(b)(2), no discharge of dredged or fill material shall be permitted if there is a practicable alternative to the proposed discharge which would have less adverse impact on the aquatic ecosystem, so long as the alternative does not have other significant adverse environmental consequences.

(1) For the purpose of this requirement, practicable alternatives include, but are not limited to:

(i) Activities which do not involve a discharge of dredged or fill material into the waters of the United States or ocean waters;

(ii) Discharges of dredged or fill material at other locations in waters of the United States or ocean waters;

(2) An alternative is practicable if it is available and capable of being done after taking into consideration cost, existing technology, and logistics in light of overall project purposes. If it is otherwise a practicable alternative, an area not presently owned by the applicant which could

reasonably be obtained, utilized, expanded or managed in order to fulfill the basic purpose of the proposed activity may be considered.

(3) Where the activity associated with a discharge which is proposed for a special aquatic site (as defined in Subpart E) does not require access or proximity to or siting within the special aquatic site in question to fulfill its basic purpose (i.e., is not "water dependent"), practicable alternatives that do not involve special aquatic sites are presumed to be available, unless clearly demonstrated otherwise. In addition, where a discharge is proposed for a special aquatic site, all practicable alternatives to the proposed discharge which do not involve a discharge into a special aquatic site are presumed to have less adverse impact on the aquatic ecosystem, unless clearly demostrated otherwise.

(4) For actions subject to NEPA, where the Corps of Engineers is the permitting agency, the analysis of alternatives required for NEPA environmental documents, including supplemental Corps NEPA documents, will in most cases provide the information for the evaluation of alternatives under these Guidelines. On occasion, these NEPA documents may address a broader range of alternatives than required to be considered under this paragraph or may not have considered the alternatives in sufficient detail to respond to the requirements of these Guidelines. In the latter case, it may be necessary to supplement these NEPA documents with this additional information.

(5) To the extent that practicable alternatives have been identified and evaluated under a Coastal Zone Management program, a Sec. 208 program, or other planning process, such evaluation shall be considered by the permitting authority as part of the consideration of alternatives under the Guidelines. Where such evaluation is less complete than that contemplated under this subsection, it must be supplemented accordingly.

(b) No discharge of dredged or fill material shall be permitted if it:

(1) Causes or contributes, after consideration of disposal site dilution and dispersion, to violations of any applicable State water quality standard;

(2) Violates any applicable toxic effluent standard or prohibition under section 307 of the Act;

(3) Jeopardizes the continued existence of species listed as endangered or threatened under the Endangered Species Act of 1973, as amended, or results in likelihood of the destruction or adverse modification of a habitat which is determined by the Secretary of Interior or Commerce, as appropriate, to be a critical habitat under the Endangered Species Act of 1973, as amended. If an exemption has been granted by the Endangered Species Committee, the terms of such exemption shall apply in lieu of this subparagraph;

(4) Violates any requirement imposed by the Secretary of Commerce to protect any marine sanctuary designated under Title III of the Marine Protection, Research, and Sanctuaries Act of 1972.

(c) Except as provided under Sec. 404(b)(2), no discharge of dredged or fill material shall be permitted which will cause or contribute to significant degradation of the waters of the United States. Findings of significant degradation related to the proposed discharge shall be based upon appropriate factual determinations, evaluations, and tests required by Subparts B and G, after consideration of Subparts C-F, with special emphasis on the persistence and permanence of the effects outlined in those subparts. Under these Guidelines, effects contributing to significant degradation considered individually or collectively, include:

(1) Significantly adverse effects of the discharge of pollutants on human health or welfare, including but not limited to effects on municipal water supplies, plankton, fish, shellfish, wildlife, and special aquatic sites.

(2) Significantly adverse effects of the discharge of pollutants on life stages of aquatic life and other wildlife dependent on aquatic ecosystems, including the transfer, concentration, and spread of pollutants or their byproducts outside of the disposal site through biological, physical, and chemical processes;

(3) Significantly adverse effects of the discharge of pollutants on aquatic ecosystem diversity, productivity, and stability. Such effects may include, but are not limited to, loss of fish and wildlife habitat or loss of the capacity of a wetland to assimilate nutrients, purify water, or reduce wave energy; or

(4) Significantly adverse effects of discharge of pollutants on recreational, aesthetic, and economic values.

(d) Except as provided under Sec. 404(b)(2), no discharge of dredged or fill material shall be permitted unless appropriate and practicable steps have been taken which will minimize potential adverse impacts of the discharge on the aquatic ecosystem. Subpart H identifies such possible steps.

Sec. 230.11 Factual determinations.

The permitting authority shall determine in writing the potential short-term or long-term effects of a proposed discharge of dredged or fill material on the physical, chemical, and biological components of the aquatic environment in light of Subparts C-F. Such factual determinations shall be used in Sec. 230.12 in making findings of compliance or non-compliance with the restrictions on discharge in Sec. 230.10. The evaluation and testing procedures described in Sec. 230.60 and Sec. 230.61 of Subpart G shall be used as necessary to make, and shall be described in, such determination. The determinations of effects of each proposed discharge shall include the following:

(a) Physical substrate determinations. Determine the nature and degree of effect that the proposed discharge will have, individually and cumulatively, on the characteristics of the substrate at the proposed disposal site. Consideration shall be given to the similarity in particle size, shape, and degree of compaction of the material proposed for discharge and the material constituting the substrate at the disposal site, and any potential changes in substrate elevation and bottom contours, including changes outside of the disposal site which may occur as a result of erosion, slumpage, or other movement of the discharged material. The duration and physical extent of substrate changes shall also be considered. The possible loss of environmental values (Sec. 230.20) and actions to minimize impact (Subpart H) shall also be considered in making these determinations. Potential changes in substrate elevation and bottom contours shall be predicted on the basis of the proposed method, volume, location, and rate of discharge, as well as on the individual and combined effects of current patterns, water circulation, wind and wave action, and other physical factors that may affect the movement of the discharged material.

(b) Water circulation, fluctuation, and salinity determinations. Determine the nature and degree of effect that the proposed discharge will have individually and cumulatively on water, current patterns, circulation including downstream flows, and normal water fluctuation. Consideration shall be given to water chemistry, salinity, clarity, color, odor, taste, dissolved gas levels, temperature, nutrients, and eutrophication plus other appropriate characteristics. Consideration shall also be given to the potential diversion or obstruction of flow, alterations of bottom contours, or other significant changes in the

hydrologic regime. Additional consideration of the possible loss of environmental values (Sec. 230.23-.25) and actions to minimize impacts (Subpart H), shall be used in making these determinations. Potential significant effects on the current patterns, water circulation, normal water fluctuation and salinity shall be evaluated on the basis of the proposed method, volume, location, and rate of discharge.

(c) Suspended particulate/turbidity determinations. Determine the nature and degree of effect that the proposed discharge will have, individually and cumulatively, in terms of potential changes in the kinds and concentrations of suspended particulate/turbidity in the vicinity of the disposal site. Consideration shall be given to the grain size of the material proposed for discharge, the shape and size of the plume of suspended particulates, the duration of the discharge and resulting plume and whether or not the potential changes will cause violations of applicable water quality standards. Consideration should also be given to the possible loss of environmental values (Sec. 230.21) and to actions for minimizing impacts (Subpart H). Consideration shall include the proposed method, volume, location, and rate of discharge, as well as the individual and combined effects of current patterns, water circulation and fluctuations, wind and wave action, and other physical factors on the movement of suspended particulates.

(d) Contaminant determinations. Determine the degree to which the material proposed for discharge will introduce, relocate, or increase contaminants. This determination shall consider the material to be discharged, the aquatic environment at the proposed disposal site, and the availability of contaminants.

(e) Aquatic ecosystem and organism determinations. Determine the nature and degree of effect that the proposed discharge will have, both individually and cumulatively, on the structure and function of the aquatic ecosystem and organisms. Consideration shall be given to the effect at the proposed disposal site of potential changes in substrate characteristics and elevation, water or substrate chemistry, nutrients, currents, circulation, fluctuation, and salinity, on the recolonization and existence of indigenous aquatic organisms or communities. Possible loss of environmental values (Sec. 230.31), and actions to minimize impacts (Subpart H) shall be examined. Tests as described in Sec. 230.61 (Evaluation and Testing), may be required to provide information on the effect of the discharge material on communities or populations of organisms expected to be exposed to it.

(f) Proposed disposal site determinations. (1) Each disposal site shall be specified through the application of these Guidelines. The mixing zone shall be confined to the smallest practicable zone within each specified disposal site that is consistent with the type of dispersion determined to be appropriate by the application of these Guidelines. In a few special cases under unique environmental conditions, where there is adequate justification to show that widespread dispersion by natural means will result in no significantly adverse environmental effects, the discharged material may be intended to be spread naturally in a very thin layer over a large area of the substrate rather than be contained within the disposal site.

(2) The permitting authority and the Regional Administrator shall consider the following factors in determining the acceptability of a proposed mixing zone:

(i) Depth of water at the disposal site;

(ii) Current velocity, direction, and variability at the disposal site;

(iii) Degree of turbulence;

(iv) Stratification attributable to causes such as obstructions, salinity or density profiles at the disposal site;

(v) Discharge vessel speed and direction, if appropriate;

(vi) Rate of discharge;

(vii) Ambient concentration of constituents of interest;

(viii) Dredged material characteristics, particularly concentrations of constituents, amount of material, type of material (sand, silt, clay, etc.) and settling velocities;

(ix) Number of discharge actions per unit of time;

(x) Other factors of the disposal site that affect the rates and patterns of mixing.

(g) Determination of cumulative effects on the aquatic ecosystem. (1) Cumulative impacts are the changes in an aquatic ecosystem that are attributable to the collective effect of a number of individual discharges of dredged or fill material. Although the impact of a particular discharge may constitute a minor change in itself, the cumulative effect of numerous such piecemeal changes can result in a major impairment of the water resources and interfere with the productivity and water quality of existing aquatic ecosystems.

(2) Cumulative effects attributable to the discharge of dredged or fill material in waters of the United States should be predicted to the extent reasonable and practical. The permitting authority shall collect information and solicit information from other sources about the cumulative impacts on the aquatic ecosystem. This information shall be documented and considered during the decision-making process. concerning the evaluation of individual permit applications, the issuance of a General permit, and monitoring and enforcement of existing permits.

(h) Determination of secondary effects on the aquatic ecosystem. (1) Secondary effects are effects on an aquatic ecosystem that are associated with a discharge of dredged or fill materials, but do not result from the actual placement of the dredged or fill material. Information about secondary effects on aquatic ecosystems shall be considered prior to the time final section 404 action is taken by permitting authorities.

(2) Some examples of secondary effects on an aquatic ecosystem are fluctuating water levels in an impoundment and downstream associated with the operation of a dam, septic tank leaching and surface runoff from residential or commercial developments on fill, and leachate and runoff from a sanitary landfill located in waters of the U.S. Activities to be conducted on fast land created by the discharge of dredged or fill material in waters of the United States may have secondary impacts within those waters which should be considered in evaluating the impact of creating those fast lands.

Sec. 230.12 Findings of compliance or non-compliance with the restrictions on discharge.

(a) On the basis of these Guidelines (Subparts C through G) the proposed disposal sites for the discharge of dredged or fill material must be:

(1) Specified as complying with the requirements of these Guidelines; or

(2) Specified as complying with the requirements of these Guidelines with the inclusion of appropriate and practicable discharge conditions (see Subpart H) to minimize pollution or adverse effects to the affected aquatic ecosystems; or

(3) Specified as failing to comply with the requirements of these Guidelines where:

(i) There is a practicable alternative to the proposed discharge that would have less adverse effect on the aquatic ecosystem, so long as such alternative does not have other significant adverse environmental consequences; or

(ii) The proposed discharge will result in significant degradation of the aquatic ecosystem under Sec. 230.10(b) or (c); or

(iii) The proposed discharge does not include all appropriate and practicable measures to minimize potential harm to the aquatic ecosystem; or

(iv) There does not exist sufficient information to make a reasonable judgment as to whether the proposed discharge will comply with these Guidelines.

(b) Findings under this section shall be set forth in writing by the permitting authority for each proposed discharge and made available to the permit applicant. These findings shall include the factual determinations required by Sec. 230.11, and a brief explanation of any adaptation of these Guidelines to the activity under consideration. In the case of a General permit, such findings shall be prepared at the time of issuance of that permit rather than for each subsequent discharge under the authority of that permit.

Subpart C -- Potential Impacts on Physical and Chemical Characteristics of the Aquatic Ecosystem

Note. -- The effects described in this subpart should be considered in making the factual determinations and the findings of compliance or non-compliance in Subpart B.

Sec. 230.20 Substrate.

(a) The substrate of the aquatic ecosystem underlies open waters of the United States and constitutes the surface of wetlands. It consists of organic and inorganic solid materials and includes water and other liquids or gases that fill the spaces between solid particles.

(b) Possible loss of environmental characteristics and values: The discharge of dredged or fill material can result in varying degrees of change in the complex physical, chemical, and biological characteristics of the substrate. Discharges which alter substrate elevation or contours can result in changes in water circulation, depth, current pattern, water fluctuation and water temperature. Discharges may adversely affect bottom-dwelling organisms at the site by smothering immobile forms or forcing mobile forms to migrate. Benthic forms present prior to a discharge are unlikely to recolonize on the discharged material if it is very dissimilar from that of the discharge site. Erosion, slumping, or lateral displacement of surrounding bottom of such deposits can adversely affect areas of the substrate outside the perimeters of the disposal site by changing or destroying habitat. The bulk and composition of the discharged material and the location, method, and timing of discharges may all influence the degree of impact on the substrate.

Sec. 230.21 Suspended particulates/turbidity.

(a) Suspended particulates in the aquatic ecosystem consist of fine-grained mineral particles, usually smaller than silt, and organic particles. Suspended particulates may enter water bodies as a result of land runoff, flooding, vegetative and planktonic breakdown, resuspension of bottom sediments, and man's activities including dredging and filling. Particulates may remain suspended in the water column for variable periods of time as a result of such factors as agitation of the water mass, particulate specific gravity, particle shape, and physical and chemical properties of particle surfaces.

(b) Possible loss of environmental characteristics and values: The discharge of dredged or fill material can result in greatly elevated levels of suspended particulates in the water column for varying lengths of time. These new levels may reduce light penetration and lower the rate of photosynthesis and the primary productivity of an aquatic area if they last long enough. Sight-dependent species may suffer reduced feeding ability leading to limited growth and lowered resistance to disease if high levels of suspended particulates persist. The biological and the chemical content of the suspended material may react with the dissolved oxygen in the water, which can result in oxygen depletion. Toxic metals and organics, pathogens, and viruses absorbed or adsorbed to fine-grained particulates in the material may become biologically available to organisms either in the water column or on the substrate. Significant increases in suspended particulate levels create turbid plumes which are highly visible and aesthetically displeasing. The extent and persistence of these adverse impacts caused by discharges depend upon the relative increase in suspended particulates above the amount occurring naturally, the duration of the higher levels, the current patterns, water level, and fluctuations present when such discharges occur, the volume, rate, and duration of the discharge, particulate deposition, and the seasonal timing of the discharge.

Sec. 230.22 Water.

(a) Water is the part of the aquatic ecosystem in which organic and inorganic constituents are dissolved and suspended. It constitutes part of the liquid phase and is contained by the substrate. Water forms part of a dynamic aquatic life-supporting system. Water clarity, nutrients and chemical content, physical and biological content, dissolved gas levels, pH, and temperature contribute to its life-sustaining capabilities.

(b) Possible loss of environmental characteristics and values: The discharge of dredged or fill material can change the chemistry and the physical characteristics of the receiving water at a disposal site through the introduction of chemical constituents in suspended or dissolved form. Changes in the clarity, color, odor, and taste of water and the addition of contaminants can reduce or eliminate the suitability of water bodies for populations of aquatic organisms, and for human consumption, recreation, and aesthetics. The introduction of nutrients or organic material to the water column as a result of the discharge can lead to a high biochemical oxygen demand (BOD), which in turn can lead to reduced dissolved oxygen, thereby potentially affecting the survival of many aquatic organisms. Increases in nutrients can favor one group of organisms such as algae to the detriment of other more desirable types such as submerged aquatic vegetation, potentially causing adverse health effects, objectionable tastes and odors, and other problems.

Sec. 230.23 Current patterns and water circulation.

(a) Current patterns and water circulation are the physical movements of water in the aquatic ecosystem. Currents and circulation respond to natural forces as modified by basin shape and cover, physical and chemical characteristics of water strata and masses, and energy dissipating factors.

(b) Possible loss of environmental characteristics and values: The discharge of dredged or fill material can modify current patterns and water circulation by obstructing flow, changing the direction or velocity of water flow, changing the direction or velocity of water flow and circulation, or otherwise changing the dimensions of a water body. As a result, adverse changes can occur in: location, structure, and dynamics of aquatic communities; shoreline and substrate erosion and depositon rates; the deposition of suspended particulates; the rate and extent of mixing of dissolved and suspended components of the water body; and water stratification.

Sec. 230.24 Normal water fluctuations.

(a) Normal water fluctuations in a natural aquatic system consist of daily, seasonal, and annual tidal and flood fluctuations in water level. Biological and physical components of such a system are either attuned to or characterized by these periodic water fluctuations.

(b) Possible loss of environmental characteristics and values: The discharge of dredged or fill material can alter the normal water-level fluctuation pattern of an area, resulting in prolonged periods of inundation, exaggerated extremes of high and low water, or a static, nonfluctuating water level. Such water level modifications may change salinity patterns, alter erosion or sedimentation rates, aggravate water temperature extremes, and upset the nutrient and dissolved oxygen balance of the aquatic ecosystem. In addition, these modifications can alter or destroy communities and populations of aquatic animals and vegetation, induce populations of nuisance organisms, modify habitat, reduce food supplies, restrict movement of aquatic fauna, destroy spawning areas, and change adjacent, upstream, and downstream areas.

Sec. 230.25 Salinity gradients.

(a) Salinity gradients form where salt water from the ocean meets and mixes with fresh water from land.

(b) Possible loss of environmental characteristics and values: Obstructions which divert or restrict flow of either fresh or salt water may change existing salinity gradients. For example, partial blocking of the entrance to an estuary or river mouth that significantly restricts the movement of the salt water into and out of that area can effectively lower the volume of salt water available for mixing within that estuary. The downstream migration of the salinity gradient can occur, displacing the maximum sedimentation zone and requiring salinity-dependent aquatic biota to adjust to the new conditions, move to new locations if possible, or perish. In the freshwater zone, discharge operations in the upstream regions can have equally adverse impacts. A significant reduction in the volume of fresh water moving into an estuary below that which is considered normal can affect the location and type of mixing thereby changing the characteristic salinity patterns. The resulting changed circulation pattern can cause the upstream migration of the salinity gradient displacing the maximim sedimentation zone. This migration may affect those organisms that are adapted to freshwater environments. It may also affect municipal water supplies.

Note. -- Possible actions to minimize adverse impacts regarding site characteristics can be found in Subpart H.

Subpart D -- Potential Impacts on Biological Characteristics of the Aquatic Ecosystem

Note. -- The impacts described in this subpart should be considered in making the factual determinations and the findings of compliance or non-compliance in Subpart B.

Sec. 230.30 Threatened and endangered species.

(a) An endangered species is a plant or animal in danger of extinction throughout all or a significant portion of its range. A threatened species is one in danger of becoming an endangered species in the foreseeable future throughout all or a significant portion of its range. Listings of threatened and endangered species as well as critical habitats are maintained by some individual States and by the U.S. Fish and Wildlife Service of the Department of the Interior (codified annually at 50 CFR Sec. 17.11). The Department of Commerce has authority over some threatened and endangered marine mammals, fish and reptiles.

(b) Possible loss of values: The major potential impacts on threatened or endangered species from the discharge of dredged or fill material include:

(1) Covering or otherwise directly killing species;

(2) The impairment or destruction of habitat to which these species are limited. Elements of the aquatic habitat which are particularly crucial to the continued survival of some threatened or endangered species include adequate good quality water, spawning and maturation areas, nesting areas, protective cover, adequate and reliable food supply, and resting areas for migratory species. Each of these elements can be adversely affected by changes in either the normal water conditions for clarity, chemical content, nutrient balance, dissolved oxygen, pH, temperature, salinity, current patterns, circulation and fluctuation, or the physical removal of habitat; and

(3) Facilitating incompatible activities.

(c) Where consultation with the Secretary of the Interior occurs under Section 7 of the Endangered Species Act, the conclusions of the Secretary concerning the impact(s) of the discharge on threatened and endangered species and their habitat shall be considered final.

Sec. 230.31 Fish, crustaceans, mollusks and other aquatic organisms in the food web.

(a) Aquatic organisms in the food web include, but are not limited to, finfish, crustaceans, mollusks, insects, annelids, planktonic organisms, and the plants and animals on which they feed and depend upon for their needs. All forms and life stages of an organism, throughout its geographic range, are included in this category.

(b) Possible loss of values: The discharge of dredged or fill material can variously affect populations of fish, crustaceans, mollusks and other food web organisms through the release of contaminants which adversely affect adults, juveniles, larvae, or eggs, or result in the establishment or proliferation of an undesirable competitive species of plant or animal at the expense of the desired resident species. Suspended particulates settling on attached or buried eggs can smother the eggs by limiting or sealing off their exposure to oxygenated water. Discharge of dredged and fill material may result in the debilitation or death of sedentary organisms by smothering, exposure to chemical contaminants in dissolved or suspended form, exposure to high levels of suspended particulates, reduction in food supply, or alteration of the substrate upon which they are dependent. Mollusks are particularly sensitive to the discharge of material during periods of reproduction and growth and development due primarily to their limited mobility. They can be rendered unfit for human consumption by tainting, by production and accumulation of toxins, or by ingestion and retention of pathogenic organisms, viruses, heavy metals or persistent synthetic organic chemicals. The discharge of dredged or fill material can redirect, delay, or stop the reproductive and feeding movements of some species of fish and crustacea, thus preventing their aggregation in accustomed places such as spawning or nursery grounds and

potentially leading to reduced populations. Reduction of detrital feeding species or other representatives of lower trophic levels can impair the flow of energy from primary consumers to higher trophic levels. The reduction or potential elimination of food chain organism populations decreases the overall productivity and nutrient export capability of the ecosystem.

Sec. 230.32 Other wildlife.

(a) Wildlife associated with aquatic ecosystems are resident and transient mammals, birds, reptiles, and amphibians.

(b) Possible loss of values: The discharge of dredged or fill material can result in the loss or change of breeding and nesting areas, escape cover, travel corridors, and preferred food sources for resident and transient wildlife species associated with the aquatic ecosystem. These adverse impacts upon wildlife habitat may result from changes in water levels, water flow and circulation, salinity, chemical content, and substrate characteristics and elevation. Increased water turbidity can adversely affect wildlife species which rely upon sight to feed, and disrupt the respiration and feeding of certain aquatic wildlife and food chain organisms. The availability of contaminants from the discharge of dredged or fill material may lead to the bioaccumulation of such contaminants in wildlife. Changes in such physical and chemical factors of the environment may favor the introduction of undesirable plant and animal species at the expense of resident species and communities. In some aquatic environments lowering plant and animal species diversity may disrupt the normal functions of the ecosystem and lead to reductions in overall biological productivity.

Note. -- Possible actions to minimize adverse impacts regarding characteristics of biological components of the aquatic ecosystem can be found in Subpart H.

Subpart E -- Potential Impacts on Special Aquatic Sites

Note. -- The impacts described in this subpart should be considered in making the factual determinations and the findings of compliance or non-compliance in Subpart B. The definition of special aquatic sites is found in Sec. 230.3(q-1).

Sec. 230.40 Sanctuaries and refuges.

(a) Sanctuaries and refuges consist of areas designated under State and Federal laws or local ordinances to be managed principally for the preservation and use of fish and wildlife resources.

(b) Possible loss of values: Sanctuaries and refuges may be affected by discharges of dredged or fill material which will:

(1) Disrupt the breeding, spawning, migratory movements or other critical life requirements of resident or transient fish and wildlife resources;

(2) Create unplanned, easy and incompatible human access to remote aquatic areas;

(3) Create the need for frequent maintenance activity;

(4) Result in the establishment of undesirable competitive species of plants and animals;

(5) Change the balance of water and land areas needed to provide cover, food, and other fish and wildlife habitat requirements in a way that modifies sanctuary or refuge management practices;

(6) Result in any of the other adverse impacts discussed in Subparts C and D as they relate to a particular sanctuary or refuge.

Sec. 230.41 Wetlands.

(a)(1) Wetlands consist of areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions.

(2) Where wetlands are adjacent to open water, they generally constitute the transition to upland. The margin between wetland and open water can best be established by specialists familiar with the local environment, particularly where emergent vegetation merges with submerged vegetation over a broad area in such places as the lateral margins of open water, headwaters, rainwater catch basins, and groundwater seeps. The landward margin of wetlands also can best be identified by specialists familiar with the local environment when vegetation from the two regions merges over a broad area.

(3) Wetland vegetation consists of plants that require saturated soils to survive (obligate wetland plants) as well as plants, including certain trees, that gain a competitive advantage over others because they can tolerate prolonged wet soil conditions and their competitors cannot. In addition to plant populations and communities, wetlands are delimited by hydrological and physical characteristics of the environment. These characteristics should be considered when information about them is needed to supplement information available about vegetation, or where wetland vegetation has been removed or is dormant.

(b) Possible loss of values: The discharge of dredged or fill material in wetlands is likely to damage or destroy habitat and adversely affect the biological productivity of wetlands ecosystems by smothering, by dewatering, by permanently flooding, or by altering substrate elevation or periodicity of water movement. The addition of dredged or fill material may destroy wetland vegetation or result in advancement of succession to dry land species. It may reduce or eliminate nutrient exchange by a reduction of the system's productivity, or by altering current patterns and velocities. Disruption or elimination of the wetland system can degrade water quality by obstructing circulation patterns that flush large expanses of wetland systems, by interfering with the filtration function of wetlands, or by changing the aquifer recharge capability of a wetland. Discharges can also change the wetland habitat value for fish and wildlife as discussed in Subpart D. When disruptions in flow and circulation patterns occur, apparently minor loss of wetland acreage may result in major losses through secondary impacts. Discharging fill material in wetlands as part of municipal, industrial or recreational development may modify the capacity of wetlands to retain and store floodwaters and to serve as a buffer zone shielding upland areas from wave actions, storm damage and erosion.

Sec. 230.42 Mud flats

(a) Mud flats are broad flat areas along the sea coast and in coastal rivers to the head of tidal influence and in inland lakes, ponds, and riverine systems. When mud flats are inundated, wind and wave action may resuspend bottom sediments. Coastal mud flats are exposed at extremely low tides and inundated at high tides with the water table at or near the surface of the substrate. The substrate of mud flats contains organic material and particles smaller in size than sand. They are either unvegetated or vegetated only by algal mats.

(b) Possible loss of values: The discharge of dredged or fill material can cause changes in water circulation patterns which may permanently flood or dewater the mud flat or disrupt periodic inundation, resulting in an increase in the rate of erosion or accretion. Such changes can deplete or

eliminate mud flat biota, foraging areas, and nursery areas. Changes in inundation patterns can affect the chemical and biological exchange and decomposition process occurring on the mud flat and change the deposition of suspended material affecting the productivity of the area. Changes may reduce the mud flat's capacity to dissipate storm surge runoff.

Sec. 230.43 Vegetated shallows.

(a) Vegetated shallows are permanently inundated areas that under normal circumstances support communities of rooted aquatic vegetation, such as turtle grass and eelgrass in estuarine or marine systems as well as a number of freshwater species in rivers and lakes.

(b) Possible loss of values: The discharge of dredged or fill material can smother vegetation and benthic organisms. It may also create unsuitable conditions for their continued vigor by: (1) changing water circulation patterns; (2) releasing nutrients that increase undesirable algal populations; (3) releasing chemicals that adversely affect plants and animals; (4) increasing turbidity levels, thereby reducing light penetration and hence photosynthesis; and (5) changing the capacity of a vegetated shallow to stabilize bottom materials and decrease channel shoaling. The discharge of dredged or fill material may reduce the value of vegetated shallows as nesting, spawning, nursery, cover, and forage areas, as well as their value in protecting shorelines from erosion and wave actions. It may also encourage the growth of nuisance vegetation.

Sec. 230.44 Coral reefs.

(a) Coral reefs consist of the skeletal deposit, usually of calcareous or silicaceous materials, produced by the vital activities of anthozoan polyps or other invertebrate organisms present in growing portions of the reef.

(b) Possible loss of values: The discharge of dredged or fill material can adversely affect colonies of reef building organisms by burying them, by releasing contaminants such as hydrocarbons into the water column, by reducing light penetration through the water, and by increasing the level of suspended particulates. Coral organisms are extremely sensitive to even slight reductions in light penetration or increases in suspended particulates. These adverse effects will cause a loss of productive colonies which in turn provide habitat for many species of highly specialized aquatic organisms.

Sec. 230.45 Riffle and pool complexes.

(a) Steep gradient sections of streams are sometimes characterized by riffle and pool complexes. Such stream sections are recognizable by their hydraulic characteristics. The rapid movement of water over a coarse substrate in riffles results in a rough flow, a turbulent surface, and high dissolved oxygen levels in the water. Pools are deeper areas associated with riffles. Pools are characterized by a slower stream velocity, a steaming flow, a smooth surface, and a finer substrate. Riffle and pool complexes are particularly valuable habitat for fish and wildlife.

(b) Possible loss of values: Discharge of dredged or fill material can eliminate riffle and pool areas by displacement, hydrologic modification, or sedimentation. Activities which affect riffle and pool areas and especially riffle/pool ratios, may reduce the aeration and filtration capabilities at the discharge site and downstream, may reduce stream habitat diversity, and may retard repopulation of the disposal site and downstream waters through sedimentation and the creation of unsuitable habitat. The discharge of dredged or fill material which alters stream hydrology may cause scouring or sedimentation of riffles and pools. Sedimentation induced through hydrological modification or as a direct result of the deposition of unconsolidated dredged or fill material may clog riffle and pool areas, destroy habitats,

and create anaerobic conditions. Eliminating pools and meanders by the discharge of dredged or fill material can reduce water holding capacity of streams and cause rapid runoff from a watershed. Rapid runoff can deliver large quantities of flood water in a short time to downstream areas resulting in the destruction of natural habitat, high property loss, and the need for further hydraulic modification.

Note. -- Possible actions to minimize adverse impacts on site or material characteristics can be found in Subpart H.

Subpart F -- Potential Effects on Human Use Characteristics

Note. -- The effects described in this subpart should be considered in making the factual determinations and the findings of compliance or non-compliance in Subpart B.

Sec. 230.50 Municipal and private water supplies.

(a) Municipal and private water supplies consist of surface water or ground water which is directed to the intake of a municipal or private water supply system.

(b) Possible loss of values: Discharges can affect the quality of water supplies with respect to color, taste, odor, chemical content and suspended particulate concentration, in such a way as to reduce the fitness of the water for consumption. Water can be rendered unpalatable or unhealthy by the addition of suspended particulates, viruses and pathogenic organisms, and dissolved materials. The expense of removing such substances before the water is delivered for consumption can be high. Discharges may also affect the quantity of water available for municipal and private water supplies. In addition, certain commonly used water treatment chemicals have the potential for combining with some suspended or dissolved substances from dredged or fill material to form other products that can have a toxic effect on consumers.

Sec. 230.51 Recreational and commercial fisheries.

(a) Recreational and commercial fisheries consist of harvestable fish, crustaceans, shellfish, and other aquatic organisms used by man.

(b) Possible loss of values: The discharge of dredged or fill materials can affect the suitability of recreational and commercial fishing grounds as habitat for populations of consumable aquatic organisms. Discharges can result in the chemical contamination of recreational or commercial fisheries. They may also interfere with the reproductive success of recreational and commercially important aquatic species through disruption of migration and spawning areas. The introduction of pollutants at critical times in their life cycle may directly reduce populations of commercially important aquatic organisms or indirectly reduce them by reducing organisms upon which they depend for food. Any of these impacts can be of short duration or prolonged, depending upon the physical and chemical impacts of the discharge and the biological availability of contaminants to aquatic organisms.

Sec. 230.52 Water-related recreation.

(a) Water-related recreation encompasses activities undertaken for amusement and relaxation. Activities encompass two broad categories of use: consumptive, e.g., harvesting resources by hunting and fishing; and non-comsumptive, e.g. canoeing and sight-seeing.

(b) Possible loss of values: One of the more important direct impacts of dredged or fill disposal is to impair or destroy the resources which support recreation activities. The disposal of dredged or fill

material may adversely modify or destroy water use for recreation by changing turbidity, suspended particulates, temperature, dissolved oxygen, dissolved materials, toxic materials, pathogenic organisms, quality of habitat, and the aesthetic qualities of sight, taste, odor, and color.

Sec. 230.53 Aesthetics.

(a) Aesthetics associated with the aquatic ecosystem consist of the perception of beauty by one or a combination of the senses of sight, hearing, touch, and smell. Aesthetics of aquatic ecosystems apply to the quality of life enjoyed by the general public and property owners.

(b) Possible loss of values: The discharge of dredged or fill material can mar the beauty of natural aquatic ecosystems by degrading water quality, creating distracting disposal sites, inducing inappropriate development, encouraging unplanned and incompatible human access, and by destroying vital elements that contribute to the compositional harmony or unity, visual distinctiveness, or diversity of an area. The discharge of dredged or fill material can adversely affect the particular features, traits, or characteristics of an aquatic area which make it valuable to property owners. Activities which degrade water quality, disrupt natural substrate and vegetational characteristics, deny access to or visibility of the resource, or result in changes in odor, air quality, or noise levels may reduce the value of an aquatic area to private property owners.

Sec. 230.54 Parks, national and historical monuments, national seashores, wilderness areas, research sites, and similar preserves.

(a) These preserves consist of areas designated under Federal and State laws or local ordinances to be managed for their aesthetic, educational, historical, recreational, or scientific value.

(b) Possible loss of values: The discharge of dredged or fill material into such areas may modify the aesthetic, educational, historical, recreational and/or scientific qualities thereby reducing or eliminating the uses for which such sites are set aside and managed.

Note. -- Possible actions to minimize adverse impacts regarding site or material characteristics can be found in Subpart H.

Subpart G -- Evaluation and Testing

Sec. 230.60 General evaluation of dredged or fill material.

The purpose of these evaluation procedures and the chemical and biological testing sequence outlined in Sec. 230.61 is to provide information to reach the determinations required by Sec. 230.11. Where the results of prior evaluations, chemical and biological tests, scientific research, and experience can provide information helpful in making a determination, these should be used. Such prior results may make new testing unnecessary. The information used shall be documented. Where the same information applies to more than one determination, it may be documented once and referenced in later determinations.

(a) If the evaluation under paragraph (b) indicates the dredged or fill material is not a carrier of contaminants, then the required determinations pertaining to the presence and effects of contaminants can be made without testing. Dredged or fill material is most likely to be free from chemical, biological, or other pollutants where it is composed primarily of sand, gravel, or other naturally occurring inert material. Dredged material so composed is generally found in areas of high current or wave energy such as streams with large bed loads or coastal areas with shifting bars and channels.

However, when such material is discolored or contains other indications that contaminants may be present, further inquiry should be made.

(b) The extraction site shall be examined in order to assess whether it is sufficiently removed from sources of pollution to provide reasonable assurance that the proposed discharge material is not a carrier of contaminants. Factors to be considered include but are not limited to:

(1) Potential routes of contaminants or contaminated sediments to the extraction site, based on hydrographic or other maps, aerial photography, or other materials that show watercourses, surface relief, proximity to tidal movement, private and public roads, location of buildings, municipal and industrial areas, and agricultural or forest lands.

(2) Pertinent results from tests previously carried out on the material at the extraction site, or carried out on similar material for other permitted projects in the vicinity. Materials shall be considered similar if the sources of contamination, the physical configuration of the sites and the sediment composition of the materials are comparable, in light of water circulation and stratification, sediment accumulation and general sediment characteristics. Tests from other sites may be relied on only if no changes have occurred at the extraction sites to render the results irrelevant.

(3) Any potential for significant introduction of persistent pesticides from land runoff or percolation;

(4) Any records of spills or disposal of petroleum products or substances designated as hazardous under section 311 of the Clean Water Act (See 40 CFR 116);

(5) Information in Federal, State and local records indicating significant introduction of pollutants from industries, municipalities, or other sources, including types and amounts of waste materials discharged along the potential routes of contaminants to the extraction site; and

(6) Any possibility of the presence of substantial natural deposits of minerals or other substances which could be released to the aquatic environment in harmful quantities by man-induced discharge activities.

(c) To reach the determinations in Sec. 230.11 involving potential effects of the discharge on the characteristics of the disposal site, the narrative guidance in Subparts C-F shall be used along with the general evaluation procedure in Sec. 230.60 and, if necessary, the chemical and biological testing sequence in Sec. 230.61. Where the discharge site is adjacent to the extraction site and subject to the same sources of contaminants, and materials at the two sites are substantially similar, the fact that the material to be discharged may be a carrier of contaminants is not likely to result in degradation of the disposal site. In such circumstances, when dissolved material and suspended particulates can be controlled to prevent carrying pollutants to less contaminated areas, testing will not be required.

(d) Even if the Sec. 230.60(b) evaluation (previous tests, the presence of polluting industries and information about their discharge or runoff into waters of the U.S., bioinventories, etc.) leads to the conclusion that there is a high probability that the material proposed for discharge is a carrier of contaminants, testing may not be necessary if constraints are available to reduce contamination to acceptable levels within the disposal site and to prevent contaminants from being transported beyond the boundaries of the disposal site, if such constraints are acceptable to the permitting authority and the Regional Administrator, and if the potential discharger is willing and able to implement such constraints. However, even if tests are not performed, the permitting authority must still determine the probable impact of the operation on the receiving aquatic ecosystem. Any decision not to test must be explained in the determinations made under Sec. 230.11.

Sec. 230.61 Chemical, biological, and physical evaluation and testing.

Note. -- The Agency is today proposing revised testing guidelines. The evaluation and testing procedures in this section are based on the 1975 Sec. 404(b)(1) interim final Guidelines and shall remain in effect until the revised testing guidelines are published as final regulations.

(a) No single test or approach can be applied in all cases to evaluate the effects of proposed discharges of dredged or fill materials. This section provides some guidance in determining which test and/or evaluation procedures are appropriate in a given case. Interim guidance to applicants concerning the applicability of specific approaches or procedures will be furnished by the permitting authority.

(b) Chemical-biological interactive effects. The principal concerns of discharge of dredged or fill material that contain contaminants are the potential effects on the water column and on communities of aquatic organisms.

(1) Evaluation of chemical-biological interactive effects. Dredged or fill material may be excluded from the evaluation procedures specified in paragraphs (b)(2) and (3) of this section if it is determined, on the basis of the evaluation in Sec. 230.60, that the likelihood of contamination by contaminants is acceptably low, unless the permitting authority, after evaluating and considering any comments received from the Regional Administrator, determines that these procedures are necessary. The Regional Administrator may require, on a case-by-case basis, testing approaches and procedures by stating what additional information is needed through further analyses and how the results of the analyses will be of value in evaluating potential environmental effects.

If the General Evaluation indicates the presence of a sufficiently large number of chemicals to render impractical the identification of all contaminants by chemical testing, information may be obtained from bioassays in lieu of chemical tests.

(2) Water column effects. (i) Sediments normally contain constituents that exist in various chemical forms and in various concentrations in several locations within the sediment. An elutriate test may be used to predict the effect on water quality due to release of contaminants from the sediment to the water column. However, in the case of fill material originating on land which may be a carrier of contaminants, a water leachate test is appropriate.

(ii) Major constituents to be analyzed in the elutriate are those deemed critical by the permitting authority, after evaluating and considering any comments received from the Regional Administrator, and considering results of the evaluation in Sec. 230.60. Elutriate concentrations should be compared to concentrations of the same constituents in water from the disposal site. Results should be evaluated in light of the volume and rate of the intended discharge, the type of discharge, the hydrodynamic regime at the disposal site, and other information relevant to the impact on water quality. The permitting authority should consider the mixing zone in evaluating water column effects. The permitting authority may specify bioassays when such procedures will be of value.

(3) Effects on benthos. The permitting authority may use an appropriate benthic bioassay (including bioaccumulation tests) when such procedures will be of value in assessing ecological effects and in establishing discharge conditions.

(c) Procedure for comparison of sites.

(1) When an inventory of the total concentration of contaminants would be of value in comparing sediment at the dredging site with sediment at the disposal site, the permitting authority may require a sediment chemical analysis. Markedly different concentrations of contaminants between the excavation and disposal sites may aid in making an environmental assessment of the proposed disposal operation. Such differences should be interpreted in terms of the potential for harm as supported by any pertinent scientific literature.

(2) When an analysis of biological community structure will be of value to assess the potential for adverse environmental impact at the proposed disposal site, a comparison of the biological characteristics between the excavation and disposal sites may be required by the permitting authority. Biological indicator species may be useful in evaluating the existing degree of stress at both sites. Sensitive species representing community components colonizing various substrate types within the sites should be identified as possible bioassay organisms if tests for toxicity are required. Community structure studies should be performed only when they will be of value in determining discharge conditions. This is particularly applicable to large quantities of dredged material known to contain adverse quantities of toxic materials. Community studies should include benthic organisms such as microbiota and harvestable shellfish and finfish. Abundance, diversity, and distribution should be documented and correlated with substrate type and other appropriate physical and chemical environmental characteristics.

(d) Physical tests and evaluation. The effect of a discharge of dredged or fill material on physical substrate characteristics at the disposal site, as well as on the water circulation, fluctuation, salinity, and suspended particulates content there, is important in making factual determinations in Sec. 230.11. Where information on such effects is not otherwise available to make these factual determinations, the permitting authority shall require appropriate physical tests and evaluations as are justified and deemed necessary. Such tests may include sieve tests, settleability tests, compaction tests, mixing zone and suspended particulate plume determinations, and site assessments of water flow, circulation, and salinity characteristics.

Subpart H -- Actions To Minimize Adverse Effects

Note. -- There are many actions which can be undertaken in response to Sec. 203.10(d) to minimize the adverse effects of discharges of dredged or fill material. Some of these, grouped by type of activity, are listed in this subpart.

Sec. 230.70 Actions concerning the location of the discharge.

The effects of the discharge can be minimized by the choice of the disposal site. Some of the ways to accomplish this are by:

(a) Locating and confining the discharge to minimize smothering of organisms;

(b) Designing the discharge to avoid a disruption of periodic water inundation patterns;

(c) Selecting a disposal site that has been used previously for dredged material discharge;

(d) Selecting a disposal site at which the substrate is composed of material similar to that being discharged, such as discharging sand on sand or mud on mud;

(e) Selecting the disposal site, the discharge point, and the method of discharge to minimize the extent of any plume;

(f) Designing the discharge of dredged or fill material to minimize or prevent the creation of standing bodies of water in areas of normally fluctuating water levels, and minimize or prevent the drainage of areas subject to such fluctuations.

Sec. 230.71 Actions concerning the material to be discharged.

The effects of a discharge can be minimized by treatment of, or limitations on the material itself, such as:

(a) Disposal of dredged material in such a manner that physiochemical conditions are maintained and the potency and availability of pollutants are reduced.

(b) Limiting the solid, liquid, and gaseous components of material to be discharged at a particular site;

(c) Adding treatment substances to the discharge material;

(d) Utilizing chemical flocculants to enhance the deposition of suspended particulates in diked disposal areas.

Sec. 230.72 Actions controlling the material after discharge.

The effects of the dredged or fill material after discharge may be controlled by:

(a) Selecting discharge methods and disposal sites where the potential for erosion, slumping or leaching of materials into the surrounding aquatic ecosystem will be reduced. These sites or methods include, but are not limited to:

(1) Using containment levees, sediment basins, and cover crops to reduce erosion;

(2) Using lined containment areas to reduce leaching where leaching of chemical constituents from the discharged material is expected to be a problem;

(b) Capping in-place contaminated material with clean material or selectively discharging the most contaminated material first to be capped with the remaining material;

(c) Maintaining and containing discharged material properly to prevent point and nonpoint sources of pollution;

(d) Timing the discharge to minimize impact, for instance during periods of unusual high water flows, wind, wave, and tidal actions.

Sec. 230.73 Actions affecting the method of dispersion.

The effects of a discharge can be minimized by the manner in which it is dispersed, such as:

(a) Where environmentally desirable, distributing the dredged material widely in a thin layer at the disposal site to maintain natural substrate contours and elevation;

(b) Orienting a dredged or fill material mound to minimize undesirable obstruction to the water current or circulation pattern, and utilizing natural bottom contours to minimize the size of the mound;

(c) Using silt screens or other appropriate methods to confine suspended particulate/turbidity to a small area where settling or removal can occur;

(d) Making use of currents and circulation patterns to mix, disperse and dilute the discharge;

(e) Minimizing water column turbidity by using a submerged diffuser system. A similar effect can be accomplished by submerging pipeline discharges or otherwise releasing materials near the bottom;

(f) Selecting sites or managing discharges to confine and minimize the release of suspended particulates to give decreased turbidity levels and to maintain light penetration for organisms;

(g) Setting limitations on the amount of material to be discharged per unit of time or volume of receiving water.

Sec. 230.74 Actions related to technology.

Discharge technology should be adapted to the needs of each site. In determining whether the discharge operation sufficiently minimizes adverse environmental impacts, the applicant should consider:

(a) Using appropriate equipment or machinery, including protective devices, and the use of such equipment or machinery in activities related to the discharge of dredged or fill material;

(b) Employing appropriate maintenance and operation on equipment or machinery, including adequate training, staffing, and working procedures;

(c) Using machinery and techniques that are especially designed to reduce damage to wetlands. This may include machines equipped with devices that scatter rather than mound excavated materials, machines with specially designed wheels or tracks, and the use of mats under heavy machines to reduce wetland surface compaction and rutting;

(d) Designing access roads and channel spanning structures using culverts, open channels, and diversions that will pass both low and high water flows, accommodate fluctuating water levels, and maintain circulation and faunal movement;

(e) Employing appropriate machinery and methods of transport of the material for discharge.

Sec. 230.75 Actions affecting plant and animal populations.

Minimization of adverse effects on populations of plants and animals can be achieved by:

(a) Avoiding changes in water current and circulation patterns which would interfere with the movement of animals;

(b) Selecting sites or managing discharges to prevent or avoid creating habitat conducive to the development of undesirable predators or species which have a competitive edge ecologically over indigenous plants or animals;

(c) Avoiding sites having unique habitat or other value, including habitat of threatened or endangered species;

(d) Using planning and construction practices to institute habitat development and restoration to produce a new or modified environmental state of higher ecological value by displacement of some or all of the existing environmental characteristics. Habitat development and restoration techniques can be used to minimize adverse impacts and to compensate for destroyed habitat. Use techniques that have been demonstrated to be effective in circumstances similar to those under consideration wherever possible. Where proposed development and restoration techniques have not yet advanced to the pilot demonstration stage, initiate their use on a small scale to allow corrective action if unanticipated adverse impacts occur.

(e) Timing discharge to avoid spawning or migration seasons and other biologically critical time periods;

(f) Avoiding the destruction of remnant natural sites within areas already affected by development.

Sec. 230.76 Actions affecting human use.

Minimization of adverse effects on human use potential may be achieved by:

(a) Selecting discharge sites and following discharge procedures to prevent or minimize any potential damage to the aesthetically pleasing features of the aquatic site (e.g. viewscapes), particularly with respect to water quality;

(b) Selecting disposal sites which are not valuable as natural aquatic areas;

(c) Timing the discharge to avoid the seasons or periods when human recreational activity associated with the aquatic site is most important;

(d) Following discharge procedures which avoid or minimize the disturbance of aesthetic features of an aquatic site or ecosystem.

(e) Selecting sites that will not be detrimental or increase incompatible human activity, or require the need for frequent dredge or fill maintenance activity in remote fish and wildlife areas;

(f) Locating the disposal site outside of the vicinity of a public water supply intake.

Sec. 230.77 Other actions.

(a) In the case of fills, controlling runoff and other discharges from activities to be conducted on the fill;

(b) In the case of dams, designing water releases to accommodate the needs of fish and wildlife.

(c) In dredging projects funded by Federal agencies other than the Corps of Engineers, maintain desired water quality of the return discharge through agreement with the Federal funding authority on scientifically defensible pollutant concentration levels in addition to any applicable water quality standards.

(d) When a significant ecological change in the aquatic environment is proposed by the discharge of dredged or fill material, the permitting authority should consider the ecosystem that will be lost as well as the environmental benefits of the new system.

Subpart I -- Planning To Shorten Permit Processing Time

Sec. 230.80 Advanced identification of disposal areas.

(a) Consistent with these Guidelines, EPA and the permitting authority, on their own initiative or at the request of any other party and after consultation with any affected State that is not the permitting authority, may identify sites which will be considered as:

(1) Possible future disposal sites, including existing disposal sites and non-sensitive areas; or

(2) Areas generally unsuitable for disposal site specification;

(b) The identification of any area as a possible future disposal site should not be deemed to constitute a permit for the discharge of dredged or fill material within such area or a specification of a disposal site. The identification of areas that generally will not be available for disposal site specification should not be deemed as prohibiting applications for permits to discharge dredged or fill material in such areas. Either type of identification constitutes information to facilitate individual or General permit application and processing.

(c) An appropriate public notice of the proposed identification of such areas shall be issued;

(d) To provide the basis for advanced identification of disposal areas, and areas unsuitable for disposal, EPA and the permitting authority shall consider the likelihood that use of the area in question for dredged or fill material disposal will comply with these Guidelines. To facilitate this analysis, EPA and the permitting authority should review available water resources management data including data available from the public, other Federal and State agencies, and information from approved Coastal Zone Management programs and River Basin Plans.

(e) The permitting authority should maintain a public record of the identified areas and a written statement of the basis for identification.

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APPENDIX B
GUIDANCE FOR EVALUATION OF EFFLUENT DISCHARGES FROM CONFINED DISPOSAL FACILITIES

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APPENDIX B - GUIDANCE FOR EVALUATION OF EFFLUENT DISCHARGES FROM CONFINED DISPOSAL FACILITIES

B1.0 INTRODUCTION

B1.1 Background

Dredged material may be placed in diked disposal areas sometimes called confined disposal facilities (CDFs). CDFs may be considered as an alternative for contaminated dredged material that is unsuitable for disposal in open water. Possible contaminant migration pathways for confined disposal facilities include effluent discharges to surface water during filling operations, surface runoff due to precipitation, leachate into groundwater, volatilization to the atmosphere, and direct uptake by plants and animals. Subsequent cycling through food webs to animal populations living in close association with the dredged material should also be considered. Each pathway may have its own standards and criteria defined by the water quality certification or other applicable laws and regulations. If standards or criteria are not met, management options may be considered including operational modifications, treatment or containment options such as covers or liners.

This appendix provides technical guidance for evaluation of the effluent pathway. Guidance for evaluation of other pathways and for management actions and control measures for CDFs is found in USACE/EPA (1992).

Dredged material may be placed in CDFs in several ways. The most common method of filling is by direct hydraulic pipeline from cutterhead dredges. Pumpout operations from hopper dredges or hydraulic reslurry from barges results in intermittent hydraulic filling. Direct mechanical placement of dredged material from barges (or possibly from trucks) can be done with equipment located at the CDF. All of these operations result in some sort of effluent discharge, defined for purposes of this manual as that material discharged directly to receiving waters during the filling operation (this would include water discharged directly over weir structures or through filter cells or retaining dikes).

A schematic of an active hydraulically filled CDF is shown in Figure B1. Dredged material hydraulically placed in a confined disposal area settles, resulting in a thickened deposit of material overlaid by a clarified supernatant. The supernatant waters are discharged from the site as effluent during active dredging operations. The effluent may contain both dissolved contaminants and suspended solids.

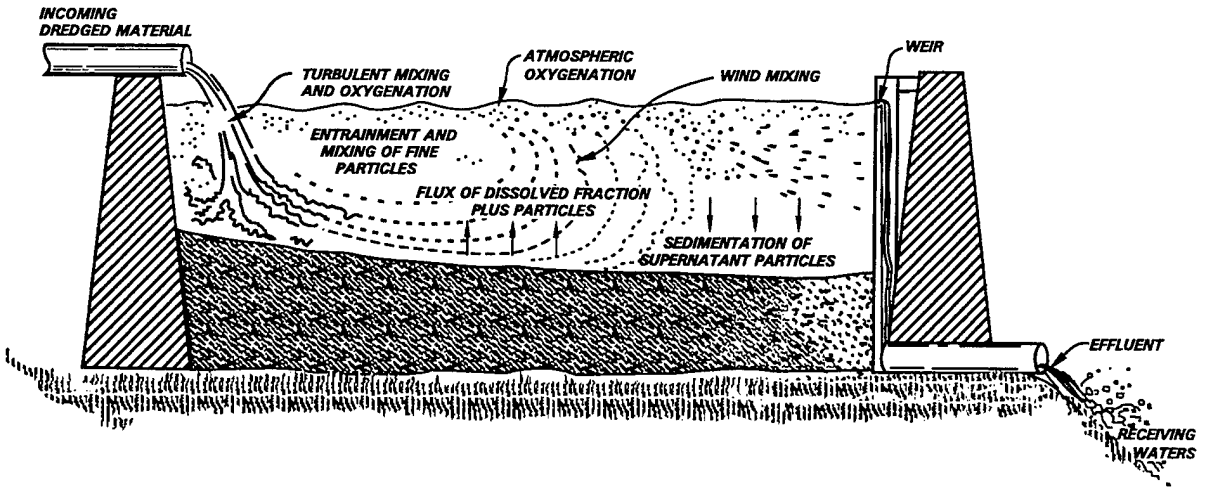


Figure B1. Schematic of Supernatant Water Interaction in an Active Confined Disposal Facility.

Supernatant waters from confined disposal sites are discharged after a retention time of up to several days. Furthermore, actual withdrawal of the supernatant is governed by the hydraulic characteristics of the ponded area and the discharge weir. Several factors influence the concentration of suspended particles present in supernatant waters. Fine particles become suspended in the disposal area water column at the point of entry due to turbulence and mixing. The suspended particles are partially removed from the water column by sedimentation. However, particle concentrations may be maintained by flow of water through the slurry mass during settling. Wind and/or surface wave action may also resuspend additional particles.

B1.2 Purpose and Scope

The purpose of this appendix is to describe procedures for evaluation of effluent discharges from CDFs. The procedures provide an estimation of potential contaminant release and/or biological effect under laboratory-simulated confined disposal conditions and consider the sedimentation behavior of dredged material, the retention time of the proposed containment area, and the physicochemical environment in ponded water during active disposal into the containment area.

B1.3 Regulatory Considerations

The quality of effluent discharged from these sites is an environmental concern and is regulated as a discharge under Section 404 of the Clean Water Act. In addition, Section 401 provides the States a certification role as to project compliance with applicable State water quality standards; effluent standards may be set as a condition of the certification.

The discharge of effluent from a CDF is defined as a dredged material discharge in 33 CFR 323.2 (d):

> ...the term "discharge of dredged material" means any addition of dredged material into, including any redeposit of dredged material within, the waters of the United States. The term includes, but is not limited to, the following: ...the runoff or overflow from a contained land or water disposal area...

Nationwide general permit 16 (33 CFR 330, Appendix A, part B (16)) authorizes the return water from an upland, contained dredged material disposal area, where the quality of the return water is controlled by the State through Section 401 Certification procedures. For all non-upland CDFs, and for all CDFs in which contaminated sediments may be discharged, an evaluation of potential contaminant impacts of the discharge will be necessary.

General permits are not intended to apply to projects involving the dredging or the discharge of contaminated material. In addition, Section 230.10 (c)(1) of the Guidelines states that no discharge of dredged material shall be permitted which will result in significant adverse impacts on municipal water supplies. Section 230.50 (a) defines municipal water supplies as surface or groundwater directed to the intake of any municipal or private water supply system. Therefore, the potential impacts of leachate into groundwater must also be considered.

There are three types of general permits issued by the USACE, nationwide permits, regional general permits and programmatic general permits. Nationwide permits are issued by the Chief of Engineers

and apply nationwide. Regional permits are issued by district and division engineers and are applicable on district or State-wide basis. Programmatic permits are issued (by the Chief of Engineers, as well as district and division engineers) to other federal, State or local agencies with the intention of providing the appropriate level of environmental protection and avoiding unnecessary duplication of effort with the agency regulatory activities at issue.

There are currently four nationwide permits that pertain to dredging and the discharge of dredged material. One authorizes the discharge and return water from confined disposal areas (provided the associated dredging is authorized pursuant to Section 10 of the River and Harbor Act of 1899); two other nationwide permits authorize the dredging and discharge, respectively, of up to 25 cubic yards of material; and a fourth authorizes maintenance dredging of existing marina basins (provided that the dredged material is deposited on uplands; return water from a confined disposal area requires separate authorization pursuant to section 404 of the Clean Water Act). As stated in the preamble to the nationwide permit regulations (FR56, 226, November 22, 1991), the USACE depends on its districts' knowledge of potentially contaminated areas and on the discretionary authority of district and division engineers to develop special conditions and/or require individual permits where contaminated sediments are present. General permits are not intended to apply to projects involving the dredging or the discharge of contaminated materials.

B1.4 Applicability

B1.4.1 Hydraulic Filling

The techniques for evaluation of effluent discharges described in this appendix are specifically designed for the case of hydraulic placement of material into CDFs with the effluent discharge occurring from an outlet pipe or weir structure or structures. Hydraulic placement can be in the form of direct pipeline inflow from cutterhead or similar hydraulic suction dredges, intermittent hydraulic placement from hopper dredge pumpout operations, or intermittent hydraulic placement by reslurrying material from barges (which may have been filled by mechanical dredges). Such placement operations would normally have an effluent discharge flowrate roughly equal to that of the inflow.

B1.4.2 Flow Through Dikes

Some CDFs may be designed to allow flow of effluent water through filter cells or permeable dike sections. The techniques described in this appendix may be applied to this case, but the influence of the filter media in adsorption of contaminants from the effluent discharge should be considered (Krizek et al., 1976).

B1.4.3 Mechanical Filling

Dredged material may be placed in some CDFs by direct mechanical means such as rehandling from barges or by truck. Although such filling operations normally involve handling relatively little free water, there may still be an effluent discharge. Also, there may be ponded water in the CDF before filling begins, especially for CDFs constructed in water. For the case of mechanical filling, the effluent discharge involves the free water which is released during the mechanical placement operation or the existing pond water which is displaced by the operation. No laboratory-developed and field-verified techniques now exist for the case of direct mechanical placement of materials in CDFs, however the procedures described here may be used in the interim for the case of mechanical placement and are considered conservative for such evaluations.

B1.4.4 Surface Runoff and Leachate

Long-term geochemical changes may occur following disposal, site dewatering, and subsequent drying of the dredged material. The quality of the surface runoff or leachate to surface water or groundwater from disposal sites after these long-term changes occur may be markedly different from that of the effluent discharged during active disposal. The techniques described in this appendix apply only to conditions during active filling of the site and do not account for long-term geochemical changes. Therefore, they should not be used to evaluate the quality of surface runoff or leachate. In accordance with 33 CFR 336.1 (b)(8) and Corps Regulatory Guidance Letter 87-8, the technical procedures contained in USACE/EPA (1992) should be used as a guide for developing the appropriate tests and evaluating surface runoff and leachate and possible management options.

B2.0 OVERVIEW OF EVALUATIONS FOR EFFLUENT DISCHARGES

The discharge of effluent from CDFs has the potential for water column effects only. Any solids in the effluent would be dispersed and mixed. Because CDFs are designed to retain virtually all of the solid fraction of dredged material, the evaluation of benthic effects is usually not applicable.

The evaluation of water column effects resulting from effluent discharges uses a tiered approach generally patterned after that for discharges of material into open water. General guidance in the main body of this manual [pertaining to Tier I evaluations (Sections 4.0 and 4.1), selection of contaminants of concern (Section 4.2), sample collection and preservation (Section 8), analytical procedures (Section 9) and general procedures for toxicity tests (Section 10)] is applicable for evaluation of effluent discharges.

B2.1 Water Quality Standards

Section 401 of the CWA requires that all Federal permits and licenses, including those for effluent or other discharges into waters of the United States, authorized pursuant to Section 404 of the CWA, must be certified as complying with applicable State water quality standards (WQS). Violations of any applicable State water quality standard apply at the edge of a State designated mixing zone.

The process for adoption of State WQS is prescribed at 40 CFR 131. States must issue, condition, deny, or waive a Water Quality Certification for activities permitted or conducted by USACE, certifying that no adverse water quality impacts will occur based on determinations of compliance with applicable State WQS which have been adopted in accordance with the above regulation. State water quality standards consist of designated uses, narrative and numeric criteria designed to support those uses, and anti-degradation provisions.

B2.2 Mixing Zones

The evaluation of effluent discharges must consider the effects of mixing and dispersion. Section 230.3(m) of the Guidelines defines the mixing zone:

> The term "mixing zone" means a limited volume of water serving as a zone of initial dilution in the immediate vicinity of a discharge point where receiving water quality may not meet quality standards or other requirements otherwise applicable to the receiving water. The mixing zone should be considered as a place where wastes and water mix and not as a place where effluents are treated.

Mixing zones are normally defined by the State regulatory agency as part of the 401 water quality certification. Detailed procedures for evaluation of mixing zones for CDF discharges are found in Appendix C.

B2.3 Basis of Evaluations

Chemical analyses are performed for contaminants that may be released from dredged material placed in CDFs and the results are compared to water quality standards for these contaminants after allowance for mixing. This provides an indirect evaluation of potential biological impacts. If water quality standards are met for all contaminants of concern, the material discharged as effluent in the water column may also be evaluated for toxicity after mixing. Toxicity tests provide information on the toxicity of contaminants not included in the water quality standards, and indicate possible

interactive effects of multiple contaminants. Bioaccumulation from the material discharged as effluent in the water column is typically considered to be of minor concern due to the short exposure time and low exposure concentrations resulting from rapid dispersion and dilution.

B2.4 Contaminant Controls

If the testing and associated analysis of the effluent pathway indicates applicable water quality standards will not be met after consideration of mixing, appropriate contaminant controls may be considered to reduce impacts to acceptable levels. Controls for effluent may include modification of the operation (e.g., use of a smaller dredge with reduced inflow rate, providing increased ponded area and depth of the CDF, or relocation of the inflow and effluent discharge points), or treatment of effluent to remove contaminants. Additional information on contaminant controls is found in USACE/EPA (1992).

B2.5 Tiered Approach

The tiered approach for evaluation of water column effects for effluent discharges from CDFs is generally patterned after that for discharges of material into open water (Section 3.1). The Tier I evaluations should be conducted as described in Section 4.0. Procedures in this appendix for evaluation of effluent discharges are performed in Tiers II and III. A flowchart illustrating the approach for evaluating potential effluent impact is shown in Figure B2. Detailed descriptions of the test procedures are given in Section B3.0. Tier IV evaluations for effluent discharges, if deemed required, would be performed considering the guidance in Section 11.3.

Tier II evaluations for effluent discharges consist of determinations of a screen relative to WQS compliance and perhaps conduct of additional water column testing. Water column testing should be conducted only if shown by the evaluation to be necessary. Water column impacts are evaluated (if necessary) by comparison of applicable water quality standards to the contaminant concentrations in the effluent discharge after consideration of mixing. Water column impact must also be evaluated by toxicity testing in Tier III when there are contaminants of concern for which applicable WQS are not available or where interactive effects are of concern.

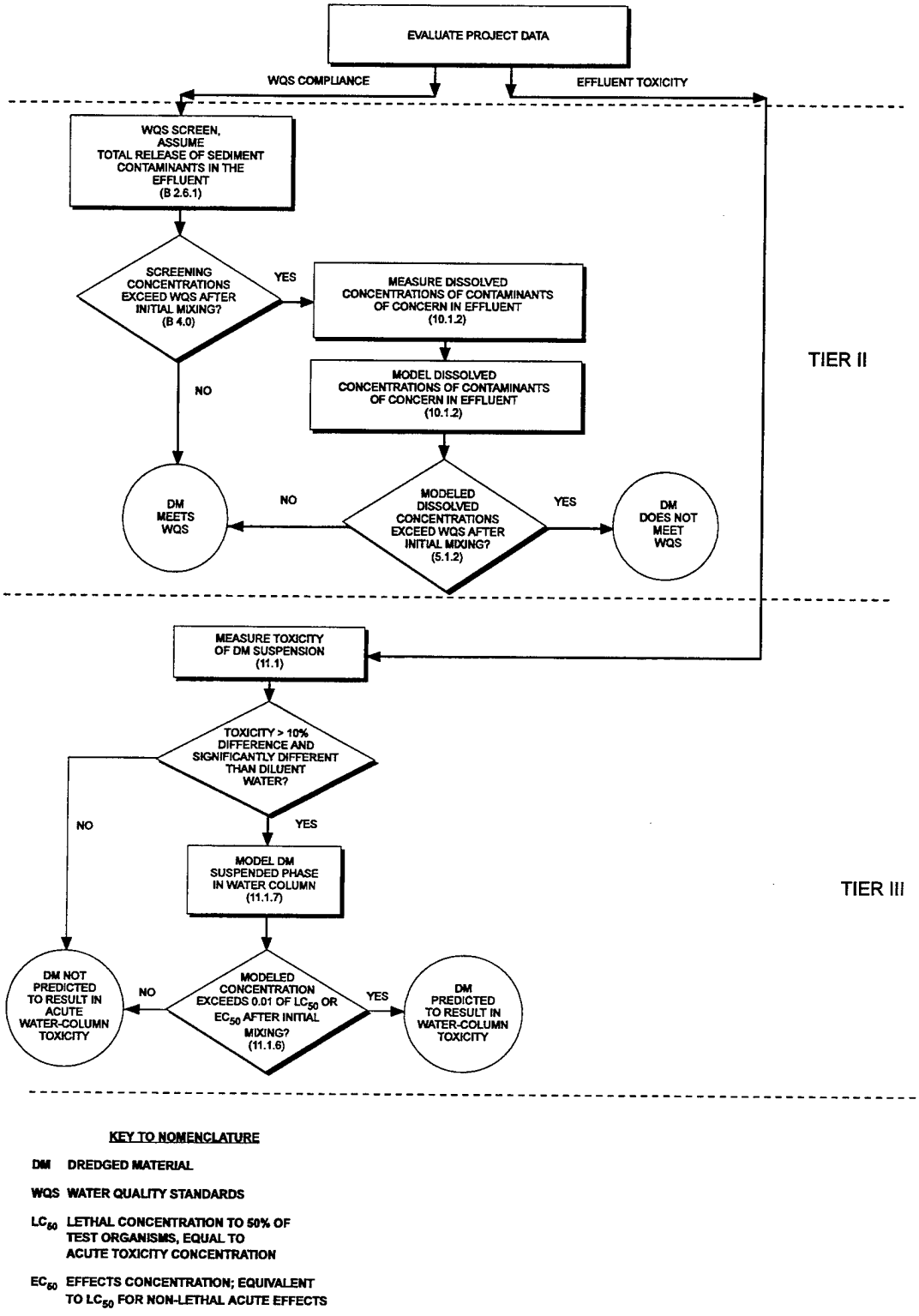


Figure B2. Flowchart Illustrating Approach for Evaluating Potential Effluent Impacts from Confined Dredged Material Disposal Areas.

B2.6 Tier II: Water Quality Evaluations

B2.6.1 Screen Relative to WQS

The screen relative to WQS determines the need for additional testing by considering the bulk concentration of contaminants in the dredged material, the mixing at the disposal site, and applicable water quality standards. If the need for additional testing is not demonstrated, the effluent discharge complies with WQS. If additional testing is needed, it is conducted according to the guidance in Section B3.0 as appropriate.

The screen involves a determination of whether the water quality standards, after consideration of mixing, would be met if the bulk concentration of contaminants present in the sediment were to be completely dissolved in the inflow water flowing into the CDF and discharged as effluent from the disposal site.

The contaminant that would require the greatest dilution is determined by calculating the dilution that would be required to meet the applicable water quality standard. To determine the dilution (D) the following equation is solved for each contaminant of concern:

$$D = [(C_s \text{ x } SS/1000) — C_{wq}] / (C_{wq} — C_{ds})$$

where
- C_s = concentration of the contaminant in the dredged material expressed as micrograms per kilogram (µg/Kg), on a dry weight basis;
- SS = suspended solids concentration in the CDF inflow expressed as grams per liter (g/L);
- 1000 = conversion factor, g to Kg;
- C_{wq} = WQS in micrograms per liter (µg/L); and
- C_{ds} = background concentration of the contaminant at the disposal site in micrograms per liter (µg/L).

The mixing zone evaluation is then made for the contaminant that would require the greatest dilution. If the concentration after mixing is below the applicable water quality standard, the effluent discharge complies with WQS. If this concentration exceeds the applicable water quality standard, additional testing must be conducted according to the guidance in Sections B2.6.2 and B3.0.

B2.6.2 Testing for Evaluation of Effluent Water Quality

The Tier II water column evaluation considers concentrations of contaminants of concern released from the dredged material (in contrast to bulk concentrations used in Section B2.6.1), after allowance

for initial mixing, compared with applicable water quality standards. The evaluation therefore requires a prediction of the CDF effluent quality.

The prediction of the quality of effluent from CDFs must account for the dissolved concentration of contaminants. An effluent elutriate procedure has been developed for this purpose (Palermo, 1986, 1988; Palermo and Thackston, 1988a and b). This test defines dissolved concentrations of contaminants in milligrams per liter, and considers the geochemical changes occurring in the disposal area during active disposal operations. Refinements and extensions of column settling test procedures (Averett et al., 1988; Montgomery et al., 1983; and Palermo and Thackston, 1988c) have also been developed to define the concentration of SS in the effluent for a given operational condition (i.e., ponded area and depth, inflow rate, and hydraulic efficiency). The column test results can be used to evaluate the turbidity of the effluent if a relationship between TSS and turbidity is defined for the sediment under consideration.

Predicted contaminant concentrations based on the results of a effluent elutriate test can be used with applicable water quality standards to determine if the discharge is in compliance with the standards after consideration of mixing. To determine the dilution (D) required to meet the standards, the following equation is solved for each contaminant of concern:

$$D = (C_{ee} - C_{wq}) / (C_{wq} - C_{ds})$$

where C_{ee} = concentration of the dissolved contaminant in the effluent elutriate in micrograms per liter (µg/L). All other terms are as previously defined in Section B2.6.1.

The mixing zone evaluation is then made for the contaminant that would require the greatest dilution. If the concentration after mixing is below the applicable water quality standard, the discharge complies with WQS. Otherwise, it does not.

B2.7 Tier III: Toxicity Evaluations

Tier III testing assesses the impacts of contaminants in the dredged material on appropriate sensitive organisms to determine if there is potential for the dredged material to have an unacceptable adverse impact. The Tier III assessment methods are toxicity tests, which use lethality as the primary endpoint because the importance of this endpoint is easily interpreted. These acute tests use organisms representative of the water column at the disposal site. The recommended procedures for water column toxicity tests for evaluation of effluent discharges are conducted in generally the same manner as those for discharges of material into open water (Section 11.1). The only exception is that the toxicity test medium is prepared using an effluent elutriate procedure.

The results of the water column toxicity tests must be interpreted considering the effects of mixing. If the concentration of dissolved plus suspended contaminants, after allowance for mixing, does not exceed 0.01 of the toxic (LC50 or EC50) concentration beyond the boundaries of the mixing zone, the discharge is predicted not to be acutely toxic to water column organisms. If the concentration of dissolved plus suspended contaminants, after allowance for mixing, exceeds 0.01 of the toxic concentration, the discharge is predicted to be acutely toxic to water column organisms.

B3.0 TESTING PROCEDURES FOR EFFLUENT DISCHARGES

This section describes the data requirements, testing procedures, and evaluation techniques necessary to predict effluent contaminant concentrations for the Tier II evaluation and to conduct water column toxicity tests for the Tier III evaluation. Example calculations are presented as appropriate.

The predictive techniques can be applied to evaluate the performance of existing sites and to design new sites. For existing sites, the technique can be used to predict the effluent quality for a given set of anticipated operational conditions (known flow and containment area size). In a similar manner, the required operational conditions for a new site (size, geometry, maximum allowable dredge size, etc.) can be determined to meet a given effluent quality requirement by comparing the predicted effluent quality for a variety of assumed operational conditions. In either case evaluation of effluent quality must be considered in conjunction with a sound design of the CDF for retention of suspended solids and initial storage of the sediments to be dredged.

B3.1 Data Requirements

Data requirements for prediction of effluent quality and effluent TSS include those pertaining to operational considerations (i.e., CDF site characteristics and dredge characteristics) and those pertaining to the properties of the dredged material (i.e., contaminant release characteristics and sedimentation characteristics). Data relating to operational considerations are usually determined by the disposal area design and by past experience in dredging and disposal activities for the project under consideration or for similar projects. Data relating to the dredged material characteristics must be obtained by sampling the sediments to be dredged and testing them. A summary of the data requirements for effluent quality prediction is given in Table B1.

B3.1.1 Disposal Area Design

When the quality of the effluent from a CDF is of concern, the design, operation, and management of the site should be carefully controlled. This includes aspects relating to both the volume required for effective sedimentation and the storage capacity of the site. Procedures for such evaluations are presented in Engineer Manual 1110-2-2-5027 (USACE, 1987), and should be considered prior to the prediction of the quality of the effluent for the project. These design procedures will determine the surface area and ponding depth required to achieve effective sedimentation, the required containment volume for storage (including required freeboard), and the proper sizing of weir structures. The prediction of the quality of the effluent described in this appendix is an extension and refinement of the design procedures. A list of data items required from the design evaluation is shown in Table B1.

The process described in Section 4.2 should identify which contaminants are of concern and which therefore should be considered for subsequent analysis in the effluent elutriate testing. The effluent elutriate tests and the column settling tests provide the remaining data required for prediction of the quality of the effluent.

B3.1.2 Sampling Requirements

Samples of channel sediment and water from the dredging site are required for conducting effluent elutriate tests and column settling tests, and toxicity tests, and for characterizing the sediment to be dredged. The level of effort, including number of sampling stations, quantity of material, and any schemes used for compositing samples, is highly project-specific. If at all possible, the sampling operations required for sediment characterization (both physical and chemical), design and evaluation of the disposal site, and conducting the effluent elutriate tests or toxicity tests should be conducted simultaneously to avoid duplication of effort. Note that water from the dredging site is used in tests for evaluation of effluent discharges. Dredging site water is used since the effluent discharge only involves a small fraction of dredged material solids and the fractionation of contaminants to the dissolved phase will be influenced primarily by that water. Note that disposal site water samples must also be taken and analyzed for evaluation of mixing. The guidance in Section 8 should be used for obtaining samples.

B3.2 Column Settling Tests

Settling tests are necessary to provide data for design or evaluation of disposal areas for retention of suspended solids. These tests are designed to define the settling behavior of a particular sediment and to provide information concerning the volumes occupied by newly placed layers of dredged material.

Table B1. Summary of Data Requirements for Prediction of the Quality of Effluent from Confined Dredged Material Disposal Areas.

Data Required	Symbol	Source of Data
Dredge inflow rate	Q_i	Project information; site design
Dredge inflow solids concentration	C_i	Project information; site design
Ponded area in disposal site	A_p	Project information; site design
Average ponding depth in disposal site and at the weir	D_p, D_{pw}	Project information; site design
Hydraulic efficiency factor	HEF	Dye tracer or theoretical determination
Effluent total suspended solids concentration	SS_{eff}	Laboratory column settling tests
Dissolved concentration of contaminant in effluent	C_{diss}	Effluent elutriate tests

* This summary includes only those data required for effluent quality prediction. It is assumed that the disposal area under consideration is designed for effective sedimentation and storage capacity. Data requirements for such design or evaluation are found in EM 1110-2-5027 (USACE, 1987).

For purposes of effluent water quality prediction, the column setting tests need only be performed if there are water quality standards for total suspended solids or turbidity. If standards exist for turbidity, a sediment-specific correlation of suspended solids and turbidity must be developed.

Sedimentation of freshwater slurries of concentration less than 100 g/L can generally be characterized as flocculent settling. As slurry concentrations are increased, the sedimentation process may be characterized as a zone settling process, in which a clearly defined interface is formed between the clarified supernatant water and the more concentrated settled material. Zone settling also occurs when the sediment/water salinity is approximately 3 ppt or greater. Flocculent settling also describes the behavior of residual suspended solids in the clarified supernatant water above the sediment/water interface for slurries exhibiting an interface. The procedures described below define the sedimentation of suspended solids under flocculent settling conditions or above the settled material/water interface under zone setting conditions. The settling test procedures consist of withdrawing samples from the settling column at various depths and times and measuring the concentrations of suspended solids.

B3.2.1 Apparatus

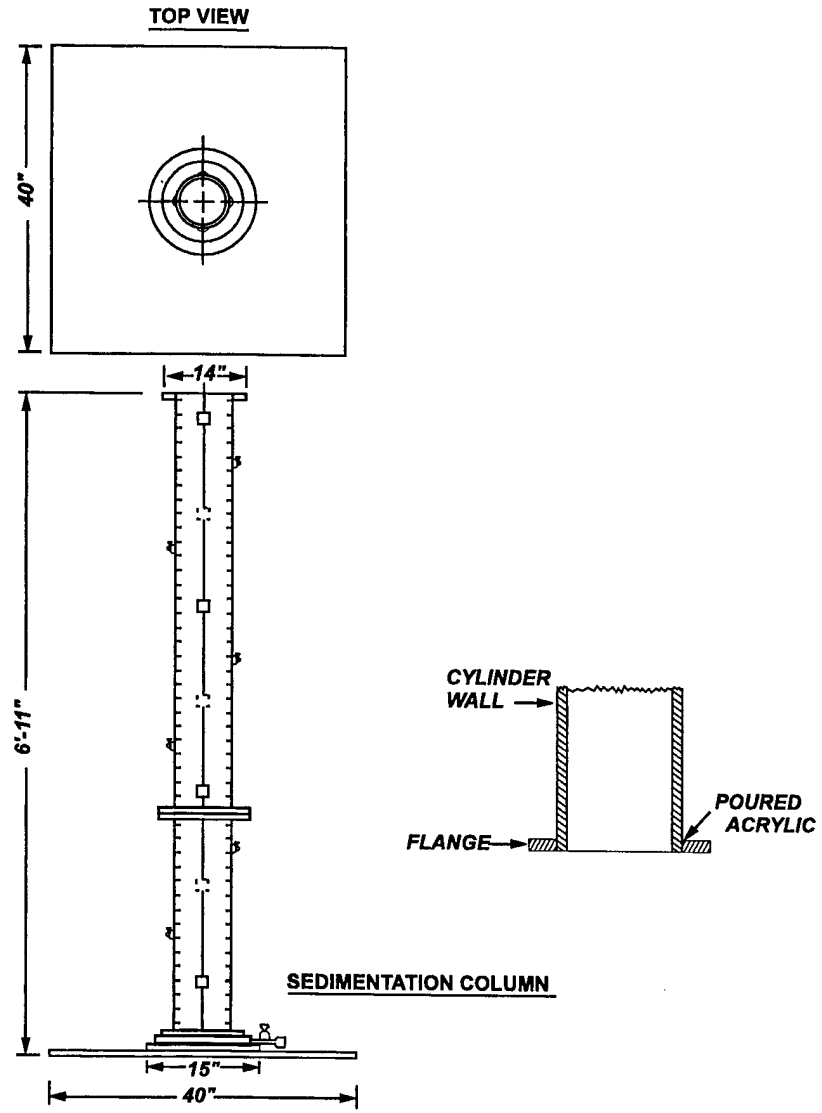
An 8-inch diameter settling column such as shown in Figure B3 is used. The test column depth should approximate the effective settling depth of the proposed disposal area. A practical limit on the depth of the test is 6 ft. The column should be at least 8 in. in diameter with interchangeable sections and with sample ports at 1/2-ft or closer intervals. The column should have provisions to bubble air from the bottom to keep the slurry mixed during the column filling period.

B3.2.2 Test Procedure

The following test procedure should be used:

Step 1. Mix the sediment slurry to a suspended solids concentration C equal to the expected concentration of the dredged material influent C_i . The slurry should be mixed in a container with sufficient volume to fill the test column. Field studies indicate that for maintenance dredging of fine-grained material, the disposal concentration will average about 150 grams per liter. This concentration should be used in the test if better data are not available.

Step 2. Pump or pour the slurry into the test column using compressed air or mechanical agitation to maintain a uniform concentration during the filling period. Any coarse material which settles to the



BILL OF MATERIAL		
QTY	MATERIAL	SIZE
1	AIR COUPLER	1/4" I.D.
1	BALL VALVE (STAINLESS STEEL)	1/4" I.D.
1	BALL VALVE (STAINLESS STEEL)	3/4" I.D.
13	SAMPLING VALVE ASSEMBLIES (STAINLESS STEEL)	3/8" I.D.
2	PIPE (STAINLESS STEEL)	1/4" I.D., 2' LONG
1	SINTERED STEEL FILTER DISK 50-80 MICRON PORE DIAMETER	1/4" THICK 8.23 DIAMETER
1	MATERIAL FOR AT LEAST 2-10" DIAMETER O-RINGS	1/4" DIA, 12' LONG
1	WATERPROOF PLYWOOD	4' x 4' x 1/2"
1	WATERPROOF PLYWOOD PLATE FOR COLUMN BASE	16" DIAMETER
8	HEX HEAD BOLT W/WASHER AND NUT	1/4" - 20 UNC x 1-1/2"
8	CARRIAGE HEAD BOLT W/WASHER AND NUT	1/4" x 3"
14	PLEXIGLASS REINFORCEMENT FOR COLUMN HOLES	3" x 3" x 1/4"
2	PLEXIGLASS FLANGE FOR BOTTOM COLUMN	1/2" THICK, 14" O.D. 8-1/2" I.D.
2	PLEXIGLASS FLANGE FOR TOP COLUMN	1/2" THICK, 14" O.D. 8-1/2" I.D.
1	PLEXIGLASS PLATE FOR COLUMN BOTTOM	1" THICK, 14" DIA
1	PLEXIGLASS CYLINDER	27" LONG, 1/4" THICK 8" I.D., 8-1/2" O.D.
1	PLEXIGLASS CYLINDER	54" LONG, 1/4" THICK 8" I.D., 8-1/2" O.D.

SUPPLIES	
MATERIAL	COMPANY
FILTER DISK	MOTT METALLURIGAICAL CORPORATION FARMINGTON INDUSTRIAL PARK FARMINGTON, CT 06032
O-RING	MISSISSIPPI RUBBER AND SPECIALITY COMPANY 819 SOUTH CONGRESS JACKSON, MS 39201
PLEXIGLASS	CADILLAC PLASTIC COMPANY/FAULKNER PLASTICS, INC. 4504 E. HILLSBORO AVE. P.O. BOX 1126 TAMPA, FL 33601

Figure B3a. Specifications for Settling Column and Plan for Sedimentation Column.

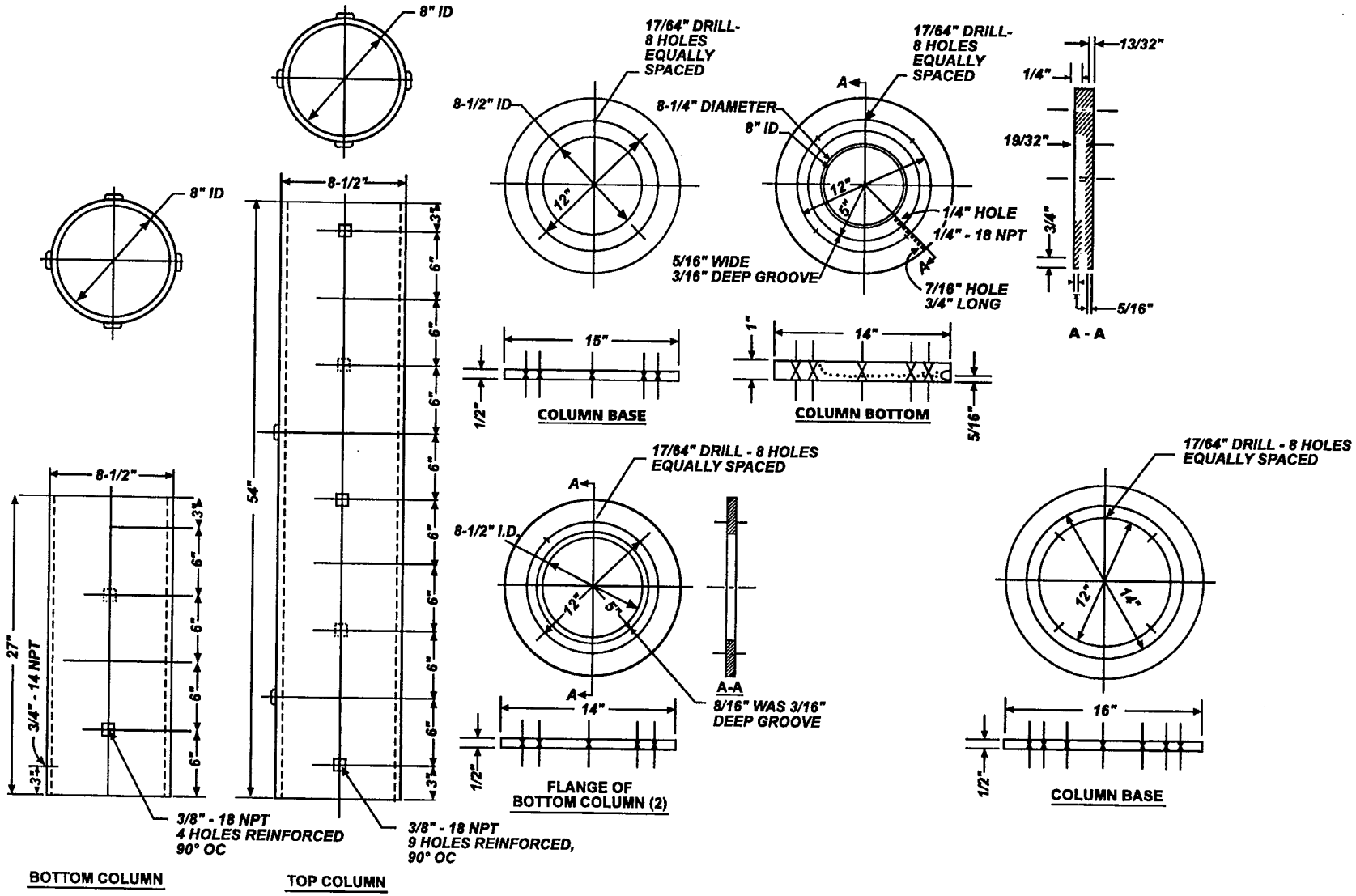


Figure B3b. Plans for Top and Bottom Columns.

bottom of the mixing container during transfer of the slurry to the test column need not be added to the column.

Step 3. When the slurry is completely mixed in the column, cut off the compressed air or mechanical agitation and immediately draw off samples at each sample port and determine their suspended solids concentration. Use the average of these values as the initial slurry concentration at the start of the test. The test is considered initiated when the first samples are drawn.

Step 4a. If an interface has not formed during the first day, flocculent settling is occurring in the entire slurry mass. Allow the slurry to settle and withdraw samples from each sampling port at regular time intervals to determine the suspended solids concentrations. Record the water surface height and time at the start of the sampling period. Analyze each sample for total suspended solids. Substantial reductions of suspended solids will occur during the early part of the test, but reductions will decrease with longer retention times. Therefore, the intervals can be extended as the test progresses. Recommended sampling intervals are 1, 2, 4, 6, 12, 24, 48 hours, etc., until the end of the test. As a rule, a 50-milliliter sample should be taken from each port. Continue the test until either an interface can be seen near the bottom of the column and the suspended solids concentration in the fluid above the interface is less than 1 gram per liter or until the suspended solids concentrations in extracted samples shows no decrease.

Step 4b. If an interface forms the first day, zone settling is occurring in the slurry below the interface, and flocculent settling is occurring in the supernatant water. For this case, samples should be extracted from all side ports above the falling interface. The first of these samples should be extracted immediately after the interface has fallen sufficiently below the uppermost port to allow extraction or sufficient sample can be withdrawn from the surface without disturbing the interface. This sample can usually be extracted within a few hours after the beginning of the test. Record the time of extraction, water surface height, and port height for each port sample taken and analyze each sample for suspended solids. As the interface continues to fall, extract samples from all ports above the interface at regular time intervals. As before, a suggested sequence of sampling intervals would be 1, 2, 4, 6, 12, 24, 48, 96 hours, etc. The samples should continue to be taken until either the suspended solids concentration of the extracted samples shows no decrease or for a maximum time of 15 days. For this case, the suspended solids in the samples should be less than 1 gram per liter, and filtration will be required to determine the concentrations. The data should be expressed in milligrams per liter for these samples. In reducing the data for this case, the concentration of the first port sample taken above the falling interface is considered the initial concentration SS_o .

B3.2.3 Data Analysis

A flocculent data analysis procedure, as outlined in the following paragraphs, is required. Example calculations are also shown in Section B3.4.

Step 1. Arrange the flocculent settling test data from the laboratory test as shown in Table B2 and compute values of the depth of sampling below the fluid surface, z. In computing the fractions of suspended solids remaining ϕ, the highest concentration of the first port samples taken is considered the initial concentration SS_o.

Step 2. Plot the values of ϕ and z using the data from the table as shown in Figure B4, forming a concentration profile diagram. Concentration-depth profiles should be plotted for each time of sample extraction.

Step 3. Use the concentration profile diagram to graphically determine percentages of suspended solids removed R for the various time intervals for the anticipated ponding depth D_{pw} (the minimum recommended ponding depth is 2 feet). This is done by graphically determining the area to the right of each concentration-depth profile and its ratio to the total area above the depth D_{pw}. The removal percentage is:

$$R = \frac{Area\ to\ right\ of\ profile}{Total\ area}\ (100)$$

Step 4. Compute the percentage P remaining as simply 100 minus the percentage removed, or:

$$P = 100 - R$$

Step 5. Compute values for suspended solids for each time of extraction as:

$$SS_t = P_t(SS_o)$$

Arrange the data as shown in Table B3.

Step 6. Plot a relationship for suspended solids concentration versus time using the value for each time of extraction, as shown in Figure B5. An exponential or power curve fitted through the data points is recommended.

Table B2. Observed Flocculent Settling Data.

Sample Extraction Time t (h)	Depth of Sample Extraction z (ft)	Suspended Solids SS (mg/L)	Fraction of Initial SS ϕ (percent)
3	0.2	93	55
3	1.0	169	100
7	1.0	100	59
7	2.0	105	62
14	1.0	45	27
14	2.0	43	25
14	3.0	50	30
24	1.0	19	11
24	2.0	18	11
24	3.0	20	12
48	1.0	15	9
48	2.0	7	4
48	3.0	14	8

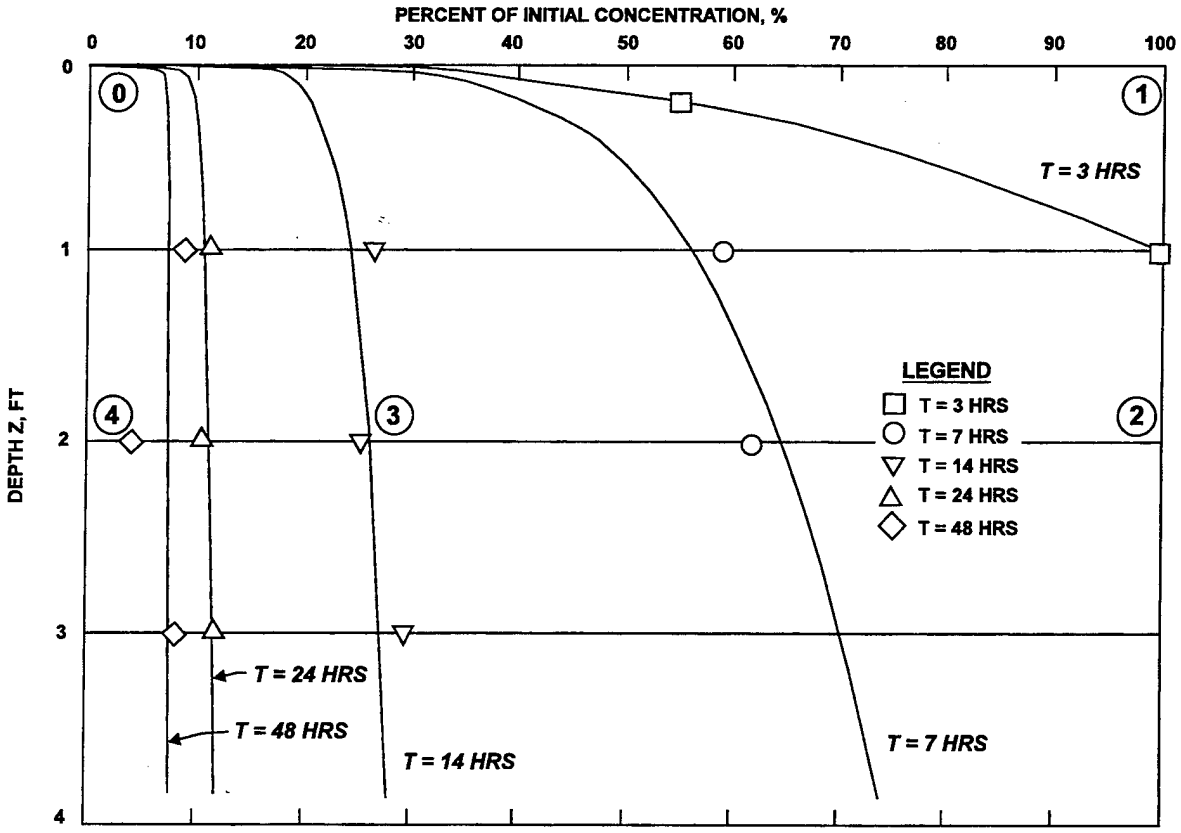


Figure B4. Concentration Profile Diagram.

Table B3. Percentage of Initial Concentration and Suspended Solids Concentrations vs. Time, Assumed Depth of Influence of 2 ft.

Sample Extraction Time t (h)	Removal Percentage, R_t	Remaining Percentage, P_t	Suspended Solids SS (mg/L)
3	14	86	145
7	47	53	90
14	78	22	37
24	90	10	17
48	94	6	10

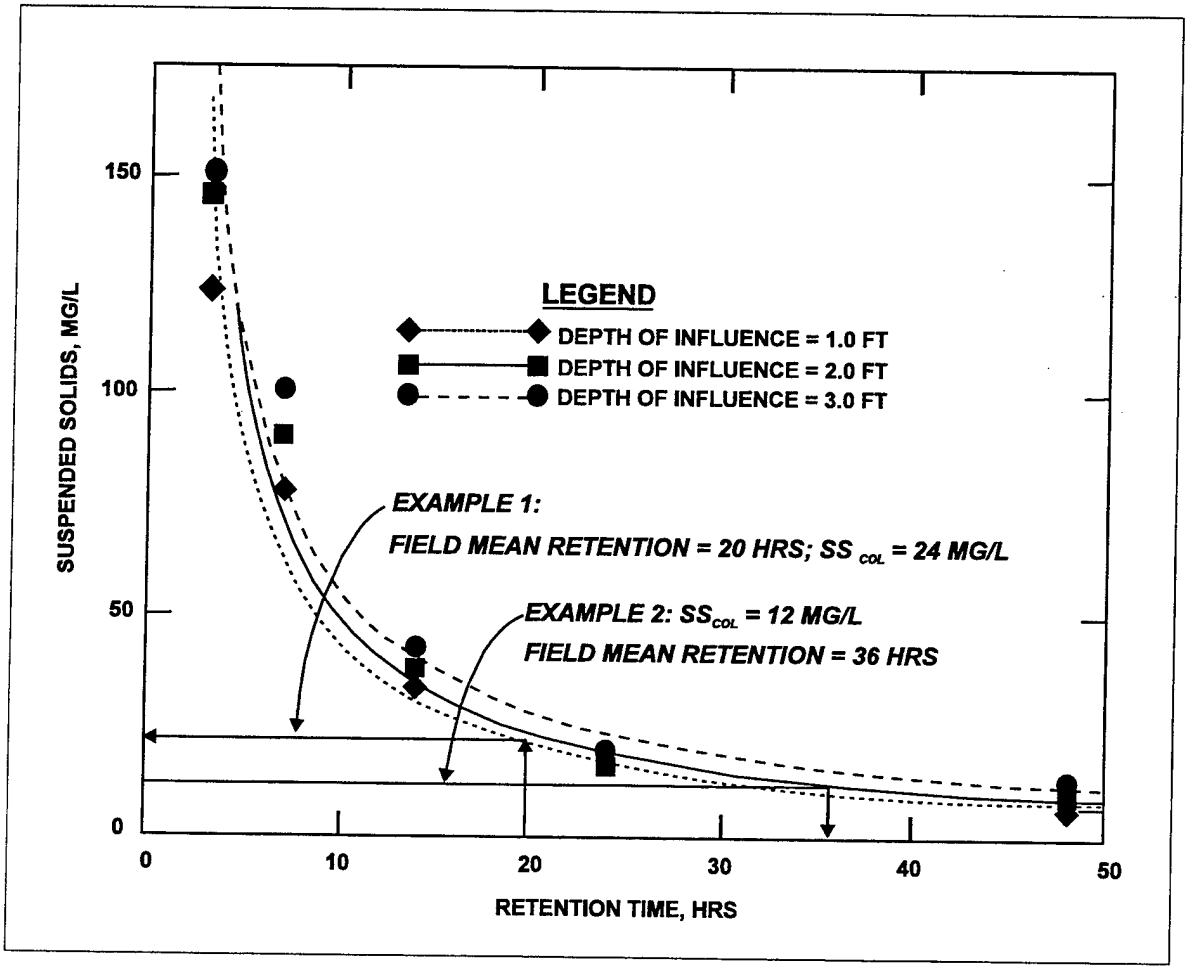


Figure B5. Plot of Supernatant Suspended Solids Concentration vs. Time from Column Settling Tests.

By repeating steps 4 through 6 for each of several values of D_{pw} , a family of curves showing suspended solids remaining versus retention time for each of several assumed ponding depths may be developed. These curves may be used for prediction of effluent suspended solids concentrations under ideal, quiescent settling conditions for any estimated ponding depth and field mean retention time. Simply enter a curve with the estimated field mean retention time T_d, and select the value of effluent suspended solids predicted by the column test SS_{col}. Guidance for determination of the field mean retention time is given in Section B3.2.5. Guidance for adjusting the value derived from the column test for anticipated resuspension is given in the Section B3.2.4.

B3.2.4 Determination of Effluent Suspended Solids Concentration

A prediction of the concentration of total suspended solids in the effluent must consider the anticipated actual mean retention time in the disposal area and must account for possible resuspension of settled material because of wind-generated turbulence. The relationship of supernatant suspended solids versus time developed from the column settling test is based on quiescent settling conditions found in the laboratory. The anticipated actual mean retention time in the disposal area under consideration can be used to determine a predicted suspended solids concentration from the relationship. This predicted value can be considered a minimum value which could only be achieved in the field if there were little or no turbulence or resuspension of settled material. However, an adjustment for anticipated resuspension is necessary for real conditions. The minimum expected value and the value adjusted for resuspension would provide a range of anticipated suspended solids concentrations for use in predicting the total concentrations of contaminants in the effluent. The value adjusted for anticipated resuspension is:

$$SS_{eff} = SS_{col} \times RF$$

where

SS_{eff} = suspended solids concentration of effluent considering anticipated resuspension, mg suspended solids/L of water

SS_{col} = suspended solids concentration of effluent as estimated from column settling tests, mg suspended solids/L of water

RF = resuspension factor selected from Table B4

Table B4 summarizes recommended resuspension factors based on comparisons of suspended solids concentrations predicted from column settling tests and field data from a number of sites with varying site conditions. For dredged material slurries exhibiting flocculent settling behavior, the concentration of particles in the ponded water is on the order of 1 g/L or higher. The resuspension resulting from normal wind conditions will not significantly increase this concentration. Therefore, an adjustment for resuspension is not required for the flocculent settling case.

Table B4. Recommended Resuspension Factors for Various Ponded Areas and Depths.

Anticipated Ponded Area	Resuspension Factor for Anticipated Average Ponded Depth	
	Less than 2 ft.	2 ft. or Greater
Less than 100 acres	2.0	1.5
Greater than 100 acres	2.5	2.0

B3.2.5 Determination of Field Mean Retention Time

Estimates of the field mean retention time for expected operational conditions are required for selecting appropriate settling times in the effluent elutriate test and for determination of suspended solids concentrations in the effluent. Estimates of the retention time must consider the hydraulic efficiency of the disposal area, defined as the ratio of mean retention time to theoretical volumetric retention time. Field mean retention time T_d can be estimated for a given flow rate and ponding conditions by applying a hydraulic efficiency correction factor (HECF) to the theoretical detention time as follows:

$$T_d = \frac{T}{(HECF)}$$

where

T_d = mean detention time, h
T = theoretical detention time, h
HECF = hydraulic efficiency correction factor (HECF > 1.0) defined as the inverse of the hydraulic efficiency

The theoretical detention time is calculated as follows:

$$T = \frac{V_p}{Q_i}\ (12.1) = \frac{A_p D_p}{Q_i}\ (12.1)$$

where

V_p = volume ponded, acre-ft
Q_i = average inflow rate, cfs
A_p = area ponded, acres
D_p = average depth of ponding, ft
12.1 = conversion factor, acre-ft/cfs to h

The hydraulic efficiency correction factor HECF can be estimated by several methods. The most accurate estimate is that made from dye tracer studies to determine T_d at the actual site under

operational conditions at a previous time, with the conditions similar to those for the operation under consideration. This approach can be used only for existing sites.

Alternatively, the ratio $T_d/T = 1/HECF$ can be estimated from the equation:

$$\frac{T_d}{T} = 0.9 \left[1 - \exp\left(-0.3 \frac{L}{W}\right)\right]$$

where L/W is the length-to-width ratio of the proposed basin. The L/W ratio can be increased greatly by the use of internal spur dikes, resulting in a higher hydraulic efficiency and a lower required total area. In the absence of dye tracer data or values obtained from other theoretical approaches, a value for HECF of 2.25 may be used based on field studies conducted at several sites (Montgomery, 1983; Montgomery et al., 1983).

B3.3 Effluent Elutriate Test Procedure

The effluent elutriate tests should be conducted, and appropriate chemical analyses should be performed, as soon as possible after sample collection. The volume of elutriate sample needed for chemical analyses will vary depending upon the number and types of chemical analyses to be conducted. The volume required for each analysis, the number of parameters measured, and the desired analytical replication will influence the total elutriate sample volume required. A 4 L cylinder is normally used for the test, and the supernatant volume available for sample extraction will vary from approximately 500 to 1,000 mL, depending on the sediment properties, settling times, and initial concentration of the slurry. It may be necessary to composite several extracted sample volumes or to use large diameter cylinders to obtain the total required volume.

B3.3.1 Apparatus

The following items are required:

a. Laboratory mixer, preferably with Teflon shaft and blades.

b. Several 4 L graduated cylinders. Larger cylinders may be used if large sample volumes are required for analytical purposes. Nalgene cylinders are acceptable for testing involving analysis of inorganic compounds such as metals and nutrients. Glass cylinders are required for testing involving analysis of organic compounds.

c. Assorted glassware for sample extraction and handling.

d. Compressed air source with deionized water trap and tubing for bubble aeration of slurry.

e. Vacuum or pressure filtration equipment, including vacuum pump or compressed air source and an appropriate filter holder capable of accommodating 47-, 105-, or 155-mm-diam filters.

f. Presoaked filters with a 0.45 μm pore-size diameter.

g. Plastic sample bottles, 500 mL capacity for storage of water and liquid phase samples for metal and nutrient analyses.

h. Wide-mouth, 1 gal capacity glass jars with Teflon-lined screw-type lids for sample mixing. These jars should also be used for sample containers when samples are to be analyzed for pesticides.

Prior to use, all glassware, filtration equipment, and filters should be thoroughly cleaned. Wash all glassware with detergent, rinse five times with tap water, place in a clean 10 percent (or stronger) HCℓ acid bath for a minimum of 4 h, rinse five times with tap water, and then rinse five times with distilled or deionized water. Soak filters for a minimum of 2 h in a 5 M HCℓ bath, and then rinse 10 times with distilled water. It is also a good practice to discard the first 50 mL of water or liquid phase filtered.

B3.3.2 Test Procedure

The step-by-step procedure for conducting the effluent elutriate test is outlined below.

Step 1 - Slurry preparation. The sediment and water from the proposed dredging site should be mixed to a concentration approximately equal to the expected average field inflow concentration. If estimates of the average field inflow concentration cannot be made based on past data, a slurry concentration of 150 g/L (dry weight basis) should be used. Predetermine the concentration of the well-mixed sediment in grams per liter (dry weight basis) by oven drying a small subsample of known volume. Each 4 L cylinder to be filled will require a mixed slurry volume of 3-3/4 L. The volumes of sediment and water to be mixed for a 3-3/4 L slurry volume may be calculated using the following expressions:

$$V_{sediment} = 3.75 \frac{C_{slurry}}{C_{sediment}}$$

and

$$V_{water} = 3.75 - V_{sediment}$$

where

$V_{sediment}$ = volume of sediment, in L
3.75 = volume of slurry for 4 L cylinder, L
C_{slurry} = desired concentration of slurry, g/L (dry weight basis)
$C_{sediment}$ = predetermined concentration of sediment, g/L (dry weight basis)
V_{water} = volume of disposal site water, in L

Step 2 - Mixing. Mix the 3-3/4 L of slurry by placing appropriate volumes of sediment and water from the proposed dredging site in a 1 gal glass jar and mixing for 5 min with the laboratory mixer. The slurry should be mixed to a uniform consistency, with no unmixed agglomerations of sediment.

Step 3 - Aeration. The prepared slurry must be aerated to ensure that oxidizing conditions will be present in the supernatant water during the subsequent settling phase. Bubble aeration is therefore used as a method of sample agitation. Pour the mixed slurry into a 4 L graduated cylinder. Attach glass tubing to the aeration source and insert the tubing to the bottom of the cylinder. The tubing can be held in place by insertion through a predrilled No. 4 stopper placed in the top of the cylinder. Compressed air should be passed through a deionized water trap, through the tubing, and bubbled through the slurry. The flow rate should be adjusted to agitate the mixture vigorously for 1 h.

Step 4 - Settling. Remove the tubing, and allow the aerated slurry to undergo quiescent settling for a time period equal to the anticipated field mean retention time, up to a maximum of 24 h. If the field mean retention time is not known, allow settling for 24 h. Guidance for estimating the field mean retention is given in Section B3.2.5.

Step 5 - Sample extraction. After the appropriate period of quiescent settling, an interface will usually be evident between the supernatant water, with a low concentration of suspended solids above, and the more concentrated settled material below the interface. Samples of the supernatant water should be extracted from the cylinder at a point midway between the water surface and interface using syringe and tubing. Care should be taken not to resuspend the settled material.

Step 6 - Sample preservation and analyses. The sample should be analyzed as soon as possible after extraction. Dissolved concentrations of desired analytes in milligrams per liter should be determined. Filtration using 0.45 μm filters should be used to obtain samples for analysis of dissolved concentrations. Samples to be analyzed for dissolved pesticides or polychlorinated biphenyls (PCBs) must be free of particles but should not be filtered due to the tendency for these materials to adsorb on the filter. However, particulate matter can be removed before analysis by high-speed

centrifugation at 10,000 times gravity using Teflon, glass, or aluminum centrifuge tubes (Fulk et al., 1975).

B3.3.3 Chemical Analyses

Chemical analyses of the effluent elutriate samples should be performed according to the guidance in Section 9.

B3.3.4 Dissolved Concentrations of Contaminants

The dissolved concentrations of chemical contaminants in the effluent elutriate are compared with water quality standards after consideration of mixing.

B3.4 Water Column Toxicity Test Procedure

The procedures for performing toxicity tests to evaluate water column effects of effluent discharges from CDFs are generally the same as those for evaluation of dredged material discharges in open water (see Section 5.1). However, the preparation of the dredged material (dissolved plus suspended contaminants) should done using the effluent elutriate procedure as described below.

The volume required for each analysis, the number of parameters measured, and the desired analytical replication will influence the total elutriate sample volume required. A 4 L cylinder is normally used for the test, and the supernatant volume available for sample extraction will vary from approximately 500 to 1,000 mL, depending on the sediment properties, settling times, and initial concentration of the slurry. It may be necessary to composite several extracted sample volumes or to use large diameter cylinders to obtain the total required volume.

B3.4.1 Apparatus

The following items are required:

a. Laboratory mixer, preferably with Teflon shaft and blades.
b. Several 4 L graduated cylinders. Larger cylinders may be used if large sample volumes are required for analytical purposes. Nalgene cylinders are acceptable for testing involving analysis of inorganic compounds such as metals and nutrients. Glass cylinders are required for testing involving analysis of organic compounds.
c. Assorted glassware for sample extraction and handling.

d. Compressed air source with deionized water trap and tubing for bubble aeration of slurry.

e. Wide-mouth, 1-gal capacity glass jars with Teflon-lined screw-type lids for sample mixing. These jars should also be used for sample containers when samples are to be analyzed for pesticides.

Prior to use, all glassware should be thoroughly cleaned. Wash all glassware with detergent, rinse five times with tap water, place in a clean bath for a minimum of 4 h, rinse five times with tap water, and then rinse five times with distilled or deionized water.

B3.4.2 Test Procedure

The step-by-step procedure for conducting the effluent elutriate test for use in toxicity tests is outlined below.

Step 1 - Slurry preparation. The sediment and water from the proposed dredging site should be mixed to a concentration approximately equal to the expected average field inflow concentration. If estimates of the average field inflow concentration cannot be made based on past data, a slurry concentration of 150 g/L (dry weight basis) should be used. Predetermine the concentration of the well-mixed sediment in grams per liter (dry weight basis) by oven drying a small subsample of known volume. Each 4 L cylinder to be filled will require a mixed slurry volume of 3-3/4 L. The volumes of sediment and water to be mixed for a 3-3/4 L slurry volume may be calculated using the following expressions:

$$V_{sediment} = 3.75 - \frac{C_{slurry}}{C_{sediment}}$$

and

$$V_{water} = 3.75 - V_{sediment}$$

where

$V_{sediment}$ = volume of sediment, in L
3.75 = volume of slurry for 4 L cylinder, L
C_{slurry} = desired concentration of slurry, g/L (dry weight basis)
$C_{sediment}$ = predetermined concentration of sediment, g/L (dry weight basis)
V_{water} = volume of dredging site water, in L

Step 2 - Mixing. Mix the 3-3/4 L of slurry by placing appropriate volumes of sediment and water from the proposed dredging site in a 1-gal glass jar and mixing for 5 min with the laboratory mixer. The slurry should be mixed to a uniform consistency, with no unmixed agglomerations of sediment.

Step 3 - Aeration. The prepared slurry must be aerated to ensure that oxidizing conditions will be present in the supernatant water during the subsequent settling phase. Bubble aeration is therefore used as a method of sample agitation. Pour the mixed slurry into a 4 L graduated cylinder. Attach glass tubing to the aeration source and insert the tubing to the bottom of the cylinder. The tubing can be held in place by insertion through a predrilled No. 4 stopper placed in the top of the cylinder. Compressed air should be passed through a deionized water trap, through the tubing, and bubbled through the slurry. The flow rate should be adjusted to agitate the mixture vigorously for 1 h.

Step 4 - Settling. Remove the tubing, and allow the aerated slurry to undergo quiescent settling for a time period equal to the anticipated field mean retention time, up to a maximum of 24 h. If the field mean retention time is not known, allow settling for 24 h. Guidance for estimating the field mean retention is given in Section B3.2.5.

Step 5 - Sample extraction. After the appropriate period of quiescent settling, an interface will usually be evident between the supernatant water, with a low concentration of suspended solids above, and the more concentrated settled material below the interface.

The liquid plus the material remaining in suspension after the settling period represents the 100 percent liquid plus suspended particulate phase. Carefully siphon the supernatant, without disturbing the settled material, and immediately use it for toxicity testing. With some very fine-grained dredged materials, it may be necessary to centrifuge the supernatant for a short time. The suspension should be clear enough at the first observation time for the organisms to be visible. The general guidance in Section 10 should be followed in performing the toxicity tests.

B4.0 EXAMPLE CALCULATIONS

B4.1 Example 1: Evaluation of Effluent Water Quality For an Existing Disposal Area

This example illustrates the evaluation of a proposed effluent discharge for an existing CDF in which effluent standards exist for dissolved contaminants and total suspended solids.

B4.1.1 Project Information

Dredged material from a maintenance project will be placed in an existing disposal site. The ponded area will be approximately 35 acres. The design indicated that the surface area is adequate for sedimentation if a minimum ponding depth of 2 ft is maintained. The dredging equipment and pumping conditions anticipated will result in a flow rate of approximately 30 cfs. A dye tracer test was

previously run at this disposal site under similar operational conditions, and the field mean retention time was 20 h. Previous sampling of inflow from the dredge pipe under similar conditions indicated that the influent solids concentration was approximately 150 g/L, which is considered a conservative maximum.

The quality of effluent must be predicted and compared with applicable water quality standards so that the acceptability of the proposed discharge may be evaluated. A field evaluation of dispersion at the disposal site determined that a dilution factor of 38 would occur in the mixing zone. For purposes of this example, copper is the parameter requiring the greatest dilution and will be used to illustrate the calculations. The water quality standard for dissolved copper at the perimeter of the mixing zone was set at 0.004 mg/L, while that for total suspended solids was set at 50 mg/L. (Note that these values are for purposes of example calculations only.)

B4.1.2 Effluent Elutriate Testing

Effluent elutriate tests were conducted on samples of sediment and disposal site water from three stations at the site. The effluent elutriate tests were run at the anticipated influent concentration, in this case 150 g/L. Sediment samples for each sampling station to be tested were homogenized, and a sediment concentration of 450 g/L was determined by oven drying a sample of known volume. The volumes of sediment and water mixed for this sample for a 3-3/4 L slurry volume were determined as:

$$V_{sediment} = 3.75 \frac{C_{slurry}}{C_{sediment}} = 3.75 \frac{150}{450} = 1.25\ L$$

and

$$V_{water} = 3.75 - V_{sediment} = 3.75 - 1.25 = 2.50\ L$$

The effluent elutriate tests were completed with the retention time used in the tests equal to the anticipated field mean retention time of 20 h. Samples were extracted for the replicate tests and analyzed for dissolved concentrations of desired parameters. The mean concentration of dissolved copper was 0.06 mg/L.

B4.1.3 Column Settling Tests

A column setting test was required because of the water quality standard for suspended solids. Samples from all stations were homogenized into a composite for the column settling test. The test used for prediction of effluent suspended solids was run at a slurry concentration of 150 g/L, equal to the anticipated influent slurry concentration. The interface was formed early in the test. Samples were extracted from settling column ports at 3, 7, 14, 24, and 48 h. Data for the solids concentrations and for various depths and extraction times are shown in Table B2.

The concentration-depth profile diagram was then constructed from the data, and is shown in Figure B4. Ratios of suspended solids removed as a function of time were then determined graphically using the step-by-step procedure described previously. Since an interface formed in the test, the slurry mass was undergoing zone settling. Therefore, the initial supernatant solids concentration SS_o was assumed to be the highest concentration of the first samples taken, 169 mg/L. The concentration-depth profile diagram was therefore constructed using 169 mg/L as ϕ = 100 percent. The lower horizontal boundaries for the area determinations corresponded to a range of assumed depths of withdrawal influence at the outlet weir, in this case 1, 2, and 3 ft. An example calculation of the removal ratio for the concentration-depth profile at t = 14 h and a depth of influence of 2 ft is:

$$R_{14} = \frac{Area\ to\ right\ of\ the\ profile}{Total\ area} = \frac{Area\ 1230*}{Area\ 1240} = 0.78$$

* Areas are designated by circled numbers in Figure B5. The areas were determined by planimeter.

The portion remaining at t = 14 h is:

$$P_{14} = 1 - R_{14} = 1 - 0.78 = 0.22$$

The value for the suspended solids remaining is:

$$SS_{14} = P_{14}\ (SS_o) = 0.22\ (169) = 37\ mg/L$$

Values at other times were determined in a similar manner. The summary data are shown in Table B3. Similar calculations for other assumed ponding depths were made. Curves were fitted to the data for total suspended solids versus retention time for depths of influence of 1, 2, and 3 ft and are shown in Figure B5.

B4.1.4 Prediction of Effluent Suspended Solids Concentration

A value for the estimated effluent suspended solids can be determined for quiescent settling conditions using the column test relationship. In this case, the field mean retention time of 20 h corresponds to a suspended solids concentration of 24 mg/L, as shown in Figure B5. This value should be adjusted for anticipated resuspension using the factors shown in Table B4. In this case, for a surface area less than 100 acres and an assumed average ponding depth of 2 ft, the resuspension factor is 1.5. The predicted total suspended solids concentration in the effluent is calculated as :

$$SS_{eff} = SS_{col} \times RF = 24\ mg/L \times 1.5 = 36\ mg/L$$

The acceptability of the discharge for suspended solids can be evaluated by comparing the estimated effluent concentration with the water quality standard, considering the appropriate mixing zone. For suspended solids, the estimated concentration of 36 mg/L is less than the water quality standard of 50 mg/L, therefore the discharge is acceptable for suspended solids prior to considering mixing.

B4.1.5 Prediction of Contaminant Concentrations

The acceptability of the proposed discharge for contaminants can be evaluated by comparing the estimated effluent concentrations with applicable water quality standards, considering an appropriate mixing zone. For a mixing zone dilution of 38 and a copper standard of 0.004 mg/L, the concentration of copper at the point of discharge must be less than 0.15 mg/L. The estimated concentration of 0.06 mg/L from the effluent elutriate test at the point of discharge is less than the limiting value of 0.15 mg/L. The discharge would therefore be acceptable.

B4.2 Example 2: Determination of Disposal Area Requirements to Meet a Given Effluent Quality Standard

This example illustrates the evaluation of a proposed effluent discharge for a new CDF in which effluent standards are defined in terms of total suspended solids. The required retention time of the new CDF to meet the standards is determined.

B4.2.1 Project Information

A disposal area is planned for contaminated sediment from a small maintenance dredging project. Dredging equipment traditionally used in the project area is capable of flow rates up to 15 cfs.

Available real estate in the project vicinity is scarce, with the maximum available area limited to 60 acres. The disposal area required to meet applicable water quality standards must be determined. The CDF design indicated that a minimum ponded surface area of 20 acres was required for effective sedimentation, assuming a flow rate of 15 cfs and an assumed minimum ponding depth of 2 ft. A mixing evaluation was conducted using a computer model and a dilution factor of 1.8 was estimated for the allowable mixing zone. The water quality standard for TSS is 10 mg/L (Note that this value is for purposes of example calculations only).

B4.2.2 Column Settling Tests

Column settling tests were performed, and the resulting concentration-depth profile was developed as was illustrated in Example 1. The column tests were run at a concentration of 150 g/L for this example. For simplicity, the test results from column tests used in the first example will also be used in this example (see Figures B4 and B5).

B4.2.3 Determination of Allowed Effluent Suspended Solids Concentration

Since this example requires determination of the disposal site characteristics necessary to meet a given water quality standard, the calculations would proceed in a manner similar to Example 1, but in reverse sequence. The concentration of effluent suspended solids required to meet water quality standards must first be determined. For a dilution of 1.8, the TSS concentration at the point of discharge must be less than 18 mg/L.

An appropriate value should be selected from Table B4 for the resuspension factor. The minimum ponding depth of 2 ft required by the site design was selected. A resuspension factor of 1.5 was selected, corresponding to an available area <100 acres and the selected ponding depth of 2 ft.

The value of 18 mg/L of SS which must be achieved at the point of discharge includes anticipated resuspension. The corresponding value for total suspended solids concentration under quiescent settling conditions is determined as:

$$S_{eff} = SS_{col} \times RF$$

or transposed,

$$SS_{col} = \frac{SS_{eff}}{RF} = \frac{18\ mg/L}{1.5} = 12\ mg/L$$

The disposal area must provide a retention time which will allow the necessary sedimentation. The required retention time to achieve 12 mg/L under quiescent settling conditions may be determined from the relationship of suspended solids versus retention time for the laboratory column. Using the concentration profile data and the selected depth of ponding at the weir of 2 ft, the relationship for suspended solids versus field mean retention was developed as was previously shown in Figure B5. Using Figure B5, 12 mg/L corresponds to a field mean retention time of 36 h. To determine the required disposal site geometry, the theoretical volumetric retention time should be used. Since no other data were available, the hydraulic efficiency correction factor was assumed to be 2.25. The theoretical volumetric retention time was calculated as:

$$T_d = \frac{T}{(HECF)}$$

or transposed,

$$T = T_d\ (HEF) = 36\ (2.25) = 81\ h$$

B4.2.4 Determination of Ponded Volume and Surface Area

The required disposal area ponded volume can now be determined using data on anticipated flow rate and the theoretical volumetric retention time. Since the dredging equipment available in the project area is capable of flow rates up to 15 cfs, the high value should be assumed.

The ponded volume required is calculated as:

$$T = \frac{V_p}{Q_i}\ (12.1)$$

or transposed,

$$V_p = \frac{TQ_i}{12.1} = \frac{81\ h \times 15\ cfs}{12.1} = 100\ acre\text{-}ft$$

A ponding depth of 2 ft is the minimum allowed. This same depth should be maintained over the entire ponded surface area and at the weir. The disposal site should therefore encompass approximately 50 acres of ponded surface area with an average depth of 2 ft if the dredge selected for the project has an effective flow rate not greater than 15 cfs. The surface area of 50 acres required to

meet the water quality standard controls the design instead of the calculated surface area of 20 acres required for effective sedimentation.

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APPENDIX C
EVALUATION OF MIXING

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C1.0 INTRODUCTION

This appendix presents a variety of techniques for evaluating the size of mixing zones for dredged material discharges. These techniques include analytical approaches and computer models for evaluation of discrete discharges from barges or hoppers, for continuous discharges from pipelines, and for effluent discharges from confined disposal facilities (CDFs). Discussions of the applicability and limitations of the techniques and stepwise procedures for performing the required calculations or applying the models are presented.

C1.1 Background

Whenever contaminant concentrations in a dredged material discharge are above water quality standards, there will be some limited initial mixing zone (or zone of dilution) in the vicinity of the discharge point where receiving water quality standards may be exceeded. The Guidelines recognize that it is not possible to set universal standards for the acceptable size of mixing zones since receiving water conditions vary so much from one location to another. The Guidelines therefore instruct that, as part of the dredging permit process, the size of any proposed mixing zone should be estimated and submitted to the permitting authority. The permitting authority must then consider receiving water conditions at the proposed site and decide if the proposed mixing-zone size is acceptable.

Many state regulatory agencies may specify a limit to mixing-zone dimensions as a condition in granting the State water quality certification. In this case the mixing zone necessary to meet applicable standards must be smaller than the specified limits.

The size of a mixing zone depends on a number of factors including the contaminant or dredged material concentrations in the discharge, concentrations in the receiving water, the applicable water quality standards, discharge density and flow rate, receiving water flow rate and turbulence, and the geometry of the discharge vessel, pipeline, or outlet structure and the receiving water boundaries. Since the maximum allowable mixing zone specified by regulatory agencies is usually on the order of hundreds of meters, the evaluation of mixing-zone sizes must necessarily be based on calculation of near-field dilution and dispersion processes.

There are a variety of possible estimation techniques for most real mixing-zone problems, but any choice of a suitable technique involves some tradeoffs. The available techniques may be thought of as ranging from sophisticated computer models, which are sometimes capable of very accurate predictions, to simple approximations that yield order-of-magnitude estimates. The most sophisticated models may not run on a microcomputer, and they may require a considerable amount of measured

data and personpower for calibration of the model to a single site. By contrast, the simplest of approximations may be made on the basis of several simplifying assumptions and hand calculations.

C1.2 Regulatory Considerations

Any evaluation of potential water column effects has to consider the effects of mixing. Section 230.3-(m) of the Guidelines defines the mixing zone as follows:

> The term "mixing zone" means a limited volume of water serving as a zone of initial dilution in the immediate vicinity of the discharge point where receiving water quality may not meet quality standards or other requirements otherwise applicable to the receiving water. The mixing zone should be considered as a place where wastes and water mix and not as a place where wastes are treated.

Further, Section 230.11(f) requires that:

> The mixing zone shall be confined to the smallest practicable zone within each specified disposal site that is consistent with the type of dispersion determined to be appropriate by the application of these Guidelines. In a few special cases under unique environmental conditions, where there is adequate justification to show that widespread dispersion by natural means will result in no significantly adverse environmental effects, the discharged material may be intended to be spread naturally in a very thin layer over a large area rather than be contained within the disposal site.

C1.3 Potential Applications of Initial Mixing

There are three potential applications of initial mixing evaluations:

a) screen to determine the need for additional water column testing under Tier II

b) evaluate dissolved contaminant concentrations by comparison with water quality standards after allowance for mixing under Tier II

c) evaluate concentrations of suspended plus dissolved constituents by comparison with toxicity test results after allowance for mixing under Tier III.

C1.3.1 Screen to Determine Need for Additional Water Column Testing

The screen determines the necessity for additional water column testing. This determination is based on a standardized calculation comparing the bulk contamination of the dredged material with water quality standards, considering the effects of initial mixing. This "worst case" approach assumes that all of the contaminants from the dredged material are released to the fluid fraction and subsequently to the water column. Mixing evaluations need only be made for the contaminant requiring the greatest dilution to meet its water quality standard. The key parameter derived from the evaluation is the maximum concentration of the contaminant in the water column at the boundary of the mixing zone. This concentration is compared with the applicable water quality standard to determine if additional water column testing is necessary. This evaluation cannot be used to predict water column impacts but only to determine the need for additional water column testing.

C1.3.2 Evaluation of Dissolved Contaminant Concentrations by Comparison with Water Quality Standards

If additional water column testing is necessary, the potential for water column impacts may be evaluated under Tier II by comparison of predicted dissolved contaminant concentrations, as determined by an elutriate test, with the water quality standards, considering the effects of mixing. This approach is used if there are water quality standards for all contaminants of concern; if these conditions are not met, the procedure in Section C1.3.3 is used. The mixing evaluation need only be made for the contaminant requiring the greatest dilution to meet its water quality standard. The key parameters derived from the model are the maximum dissolved concentration of the contaminant at the boundary of the mixing zone. This concentration is compared to the applicable water quality standard to determine if the discharge complies with the Guidelines.

C1.3.3 Evaluation of Concentrations of Suspended Plus Dissolved Constituents by Comparison with Toxicity Test Results

If additional water column testing is necessary, the potential for water column impact may be evaluated under Tier III by comparison of predicted concentrations of the suspended plus dissolved constituents of the dredged material with toxicity test results, considering the effects of mixing. For this case, the dilution of the dredged-material elutriate expressed as a percent of the initial volume of disposed fluid in a given volume of water column is calculated. The key parameters derived from the evaluation are the maximum concentration of dredged-material elutriate in the water column at the

boundary of the mixing zone. These concentrations are compared to 0.01 of the LC_{50} or EC_{50} as determined by toxicity tests to determine if the discharge complies with the Guidelines.

C1.4 Physical Characteristics of Dredged Material Discharges

Knowledge of the physical characteristics of dredged material discharges is necessary for proper selection of a technique or model for evaluation of initial mixing. Dredged material can be placed in open-water sites using direct pipeline discharge, direct mechanical placement, or release from hopper dredges or scows. Discharges of effluent from CDFs can be introduced to the receiving waters in a variety of ways including direct pipeline outfalls or open channels. For purposes of evaluation of initial mixing, barges or hopper dredge discharges are discrete discharges, while direct discharge from a pipeline dredge or CDF effluent should be considered continuous discharges.

C1.4.1 Barge Discharge

Bucket or clamshell dredges remove the sediment being dredged at nearly its *in situ* density and place it on a barge or scow for transportation to the disposal area. Although several barges may be used so that the dredging is essentially continuous, disposal occurs as a series of discrete discharges. Barges are designed with bottom doors or with a split-hull, and the contents may be emptied within seconds, essentially as an instantaneous discharge. Often sediments dredged by clamshell remain in fairly large consolidated clumps and reach the bottom in this form. Whatever its form, the dredged material descends rapidly through the water column to the bottom, and only a small amount of the material remains suspended. Clamshell dredge operations may also be used for direct material placement adjacent to the area being dredged. In these instances, the material also falls directly to the bottom as consolidated clumps.

C1.4.2 Hopper Dredge Discharge

The characteristics and operation of hopper dredges result in a mixture of water and solids stored in the hopper for transport to the disposal site. At the disposal site, hopper doors in the bottom of the ship's hull are opened, and the entire hopper contents are emptied in a matter of minutes; the dredge then returns to the dredging site to reload. This procedure produces a series of discrete discharges at intervals of perhaps one to several hours. Upon release from the hopper dredge at the disposal site, the dredged material falls through the water column as a well-defined jet of high-density fluid which may contain blocks of solid material. Ambient water is entrained during descent. After it hits bottom,

most of the dredged material comes to rest. Some material enters the horizontally spreading bottom surge formed by the impact and is carried away from the impact point until the turbulence of the surge is sufficiently reduced to permit its deposition.

C1.4.3 Pipeline Dredge Discharge

Pipeline dredges are commonly used for open-water disposal adjacent to channels. Material from this dredging operation consists of a slurry with solids concentration ranging from a few grams per liter to several hundred grams per liter. Depending on material characteristics, the slurry may contain clay balls, gravel, or coarse sand material. This coarse material quickly settles to the bottom. The mixture of dredging site water and finer particles has a higher density than the disposal site water and therefore can descend to the bottom forming a fluid mud layer. Continuing the discharge may cause the fluid mud layer to spread. There will be a vertical gradient of fine suspended solids forming a turbidity layer above the fluid mud layer, created by the discharge momentum and resulting turbulence and entrainment of disposal site water into the discharge plume. The suspended solids concentration of the fluid mud layer is typically 10 g/L or greater while the overlying turbidity layer is defined as less than 10 g/L. Characteristics of the plume are determined by: discharge rate, characteristics of the slurry (both water and solids), water depth, currents, meteorological conditions, salinity of receiving water, and discharge configuration.

C1.4.4 Confined Disposal Facility (CDF) Effluent Discharge

Dredged material hydraulically placed in a confined disposal area settles, resulting in a thickened deposit of material overlaid by a clarified supernatant. The supernatant waters are discharged from the site as effluent during active dredging operations. The effluent may contain both dissolved contaminants and suspended colloidal particles with associated (adsorbed or held by ion exchange) contaminants. Supernatant waters from confined disposal sites are discharged after a retention time of up to several days. Furthermore, actual withdrawal of the supernatant is governed by the hydraulic characteristics of the ponded area and the discharge weir. The effluent suspended solids concentration is typically less than 100 mg/L for sediments dredged from estuarine environments and less than a few grams per liter for sediments dredged from freshwater environments.

C1.5 Applicability of Models and Techniques

C1.5.1 General Considerations

Equations can be derived from a simplistic approach to the problem of estimating mixing-zone size that make it possible to use a combination of empirical and analytical solutions. However, the simplifications that make the calculations easily manageable are somewhat restrictive, and a more advanced set of similar empirical and analytical solutions could be used to estimate mixing-zone sizes under more complex conditions. The more advanced analytical solutions involve many more computations, and for this reason they are more easily dealt with by use of a computer. The simplicity and limited data requirements of analytical solutions make them an attractive tool. However, analytical solutions cannot be used for receiving water where there are complex hydrodynamic conditions, nor can they be applied under dynamic (unsteady) flow conditions. Where these conditions exist, a numerical model must be used, and numerical dispersion models are not susceptible to hand calculation. In addition to requiring a computer solution technique, numerical models generally require a much more detailed set of input data, and the collection of such data can be expensive.

No models have been identified that are suitable for a broad range of mixing zone conditions, and there are no readily available models suitable for modeling the first few hundred metres downstream from the discharge point. This is because the overwhelming majority of computer models are concerned with far-field solutions where concentrations can be adequately described by a two-dimensional or a one-dimensional model and the initial characteristics of the discharge are relatively unimportant. These models are generally inadequate in the immediate vicinity of a discharge, where a three-dimensional description of concentrations is often necessary and where the initial characteristics of the discharge can be highly significant. Within the first few hundred metres of the discharge, there are several different processes that may be significant, so a general model must be able to estimate each of the processes (for example, momentum, buoyancy, dispersion) and to identify the zones within which the processes are dominant. A general mixing-zone model must therefore be a series of submodels, each of which can handle a zone that is dominated by one of the principal mixing processes. The sub-models must be capable of determining the limits of their applicable zones and passing concentration values at these limits on to other submodels so that the entire mixing zone may be estimated. The following tabulation presents a summary of the steady-state physical processes that might be suitable for inclusion as submodels in a general mixing-zone model. Sources that presently seem to present the most promising empirical and analytical solutions to these sub-model processes are also presented in the tabulation.

Physical Process to be Handled by a Submodel	Source
Momentum and/or buoyancy-dominated jets	Zeller et al. (1971) Motz and Benedict (1972) Buhler and Hauenstein (1981) Jirka et al. (1981) Wright (1984) Doneckar and Jirka (1990)
3-dimensional dispersion in receiving water	Prakash (1977) Fischer et al. (1979) Johnson et al. (1994) King (1992)
2-dimensional vertically averaged dispersion	Stefan and Gulliver (1978) Paily and Sayre (1978) Gowda (1984a, b) Thomas and McAnally (1990)

C1.5.2 Considerations for Tidally Influenced Rivers and Estuaries

The assumptions necessary for evaluation of mixing are more difficult to satisfy in estuaries and the tidally influenced portions of rivers. The assumption that velocities in the water body near the mixing zone can be represented by a single mean velocity parallel to the bank is usually a reasonable one in the non-tidally influenced portion of a river. However, it is not always acceptable in estuaries. Typically the downstream section of an estuary exhibits horizontal circulation patterns, so that the horizontal water velocity and direction vary with distance parallel to the bank, distance perpendicular to the bank, and time. Under these conditions, water near the mixing zone may not always travel parallel to the bank. Therefore, simple mixing-zone equations may not be applicable to the wide, open low-velocity sections of estuaries.

Also, mixing-zone equations are not theoretically applicable as the mean velocity tends to zero. This is because the equations are dependent upon the process of advection, which does not exist in the absence of a flow velocity, and also because the primary source of dispersion is assumed to be the turbulence caused by the horizontal movement of water. However, in a real water body, as the velocity tends to zero, the primary sources of turbulence and dispersion are the wind and waves.

The rate of change of water velocity due to tidal effects can also cause problems. The time taken for material to travel the length of the mixing zone should be an order of magnitude smaller than the time taken for a 10-percent change in the mean water velocity. It may be possible to satisfy this condition

in a river, but it will probably not be possible to do so in most estuaries during a significant portion of the tidal cycle.

Another potential difficulty in estuaries is the phenomenon of stratification. Estuaries with low water velocities sometimes have a layer of relatively fresh water near the surface with a much more saline denser layer of water near the bottom and with quite a distinct interface between the two layers. The abrupt change of density at the interface tends to inhibit vertical mixing through the entire depth of the water column.

C1.5.3 Recommended Models and Techniques

Several models and approaches for evaluation of initial mixing are provided in this appendix. Table C-1 provides a summary of the characteristics of the various types of dredged material discharges, hydrodynamic environments, and the models recommended for use in evaluation of initial mixing for those conditions. Descriptions of each of the models and details on applying the models are provided in the following sections of this appendix.

Table C-1. Summary of Discharge Types, Hydrodynamic Conditions, and Applicable Models and Methods for Evaluation of Initial Mixing.

Type of Discharge	Characteristics of Discharge	Near-Field Effects	Applicable Model or Technique	Model Hydrodynamics	Section
BARGE	Discrete	Strong	STFATE	Steady Non-uniform	C2.0
HOPPER	Semi-Discrete	Moderate	STFATE	Steady Non-uniform	C2.0
PIPELINE	Continuous	Moderate	CD-CORMIX[1]	Steady Uniform	C3.0
			TABS[2]	Unsteady Non-uniform	C5.0
CDF EFFLUENT	Continuous	Weak	MacIntyre	Steady Uniform	C4.0
			CORMIX	Steady Uniform	C3.0
			TABS[2]	Unsteady Non-uniform	C5.0
			Dilution Volume Method	Steady Uniform	C6.0

[1] CD-CORMIX has not been developed and verified for national application. However, the fundamental processes contained in CD-CORMIX are applicable for continuous pipeline discharges and this model is currently under investigation for future use.

[2] TABS has not been developed and verified for national application for the indicated discharges. However, the fundamental far-field processes contained in TABS are applicable for the indicated discharges and this model can be adapted for use on a regional basis. Note that the TABS model computes far-field effects only. Some independent near-field analysis is usually required.

C2.0 SHORT TERM FATE MODEL FOR OPEN WATER BARGE AND HOPPER DISCHARGES (STFATE)

C2.1 Introduction

The model described in this section is the STFATE (Short-Term FATE of dredged material disposal in open water) model (Johnson et al., 1994) developed from the DIFID (Disposal From an Instantaneous Discharge) model originally prepared by Koh and Chang (1973). This model is used for discrete discharges from barges and hoppers. STFATE is a module of the Automated Dredging and Disposal Alternatives Management System (ADDAMS) (Schroeder and Palermo, 1990) and can be run on DOS-based personal computers (PC) having 80386 or higher processors with math coprocessors. ADDAMS is an interactive computer-based design and analysis system in the field of dredged-material management. The general goal of 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.

An executable version of the STFATE model for use on IBM-compatible microcomputers can be downloaded from the Internet web site http://www.epa.gov/OST/pubs/ITM.html (see Section C2.6.2). The model is appropriate for instantaneous discharges from barges or scows and sequential discharges from hopper dredges.

C2.2 Theoretical Basis

The behavior of the material during disposal is assumed to be separated into three phases: convective descent, during which the disposal cloud falls under the influence of gravity and its initial momentum is imparted by gravity; dynamic collapse, occurring when the descending cloud either impacts the bottom or arrives at a level of neutral buoyancy where descent is retarded and horizontal spreading dominates; and passive transport-dispersion, commencing when the material transport and spreading are determined more by ambient currents and turbulence than by the dynamics of the disposal operation. Figure C-1 illustrates these phases.

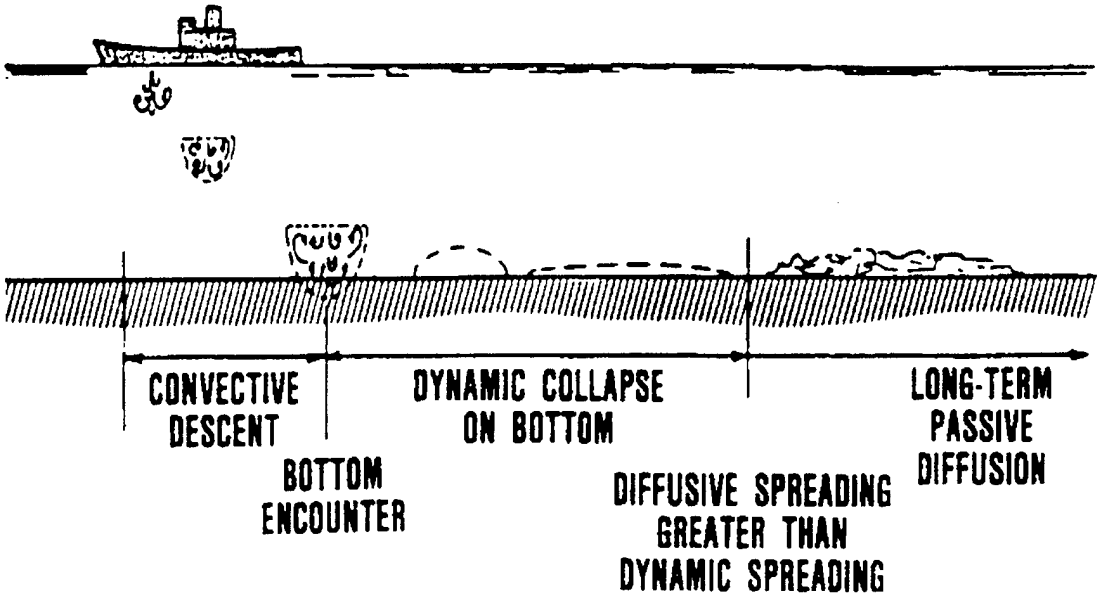


Figure C-1. Illustration of Placement Processes.

C2.2.1 Convective Descent

In STFATE, multiple convecting clouds that maintain a hemispherical shape during convective descent are assumed to be released. By representing the disposal as a sequence of convecting clouds released at a constant time interval during the total time required for the material to leave the disposal vessel, real disposal operations can be more accurately simulated. For example, a moving hopper dredge disposal can be modeled by assuming that the material in each bin convects downward as one cloud. In addition, through the use of multiple convecting clouds with varying characteristics the consolidation that often occurs in scows or barges can be accounted for more accurately. Since the solids concentration in discharged dredged material is usually low, each cloud is expected to behave as a dense liquid; thus, a basic assumption is that a buoyant thermal analysis is appropriate. The equations governing the motion are those for conservation of mass, momentum, buoyancy, solid particles, and vorticity. These equations are straightforward statements of conservation principles; details are presented in Koh and Chang (1973) and Brandsma and Divoky (1976). It should be noted that the entrainment coefficient associated with the entrainment of ambient fluid into a descending hemispherical cloud is assumed to vary smoothly between its value for a vortex ring and the value for

turbulent thermals. Model results are relatively sensitive to the entrainment coefficient, which in turn is dependent upon the material being disposed (the higher the moisture content, the larger the value of the entrainment coefficient). Laboratory studies by Bowers and Goldenblatt (1978) resulted in analytical expressions for the entrainment, drag, and added mass coefficients as functions of the moisture content. These have been incorporated into STFATE. As these clouds move downward, material and fluid with dissolved contaminants may be stripped away. Stripped material is handled through the concept of Gaussian clouds discussed below. The amount of material stripped away and stored in the Gaussian clouds is computed as a coefficient times the downward velocity of the cloud times the cloud surface area. The value of the "stripping" coefficient is selected so that approximately 2-5 percent of the total volume of fine material is stripped away at disposal sites of 100 ft or less. Based upon field data collected by Bokuniewicz et al. (1978), this will result in the amount of stripped material being on the conservative side.

C2.2.2 Dynamic Collapse

Whether by disposal from a split-hull barge or scow or discharge from a multi-bin hopper dredge, the disposed material cloud grows during convective descent as a result of entrainment. Eventually, either the material reaches the bottom, or the density difference between the discharged material and the ambient water column becomes small enough for a position of neutral buoyancy to be assumed. In either case, the vertical motion is arrested and a dynamic horizontal spreading occurs.

The basic shape assumed for each collapsing cloud is an oblate spheroid if collapse occurs in the water column, whereas a general ellipsoid is assumed for collapse on a sloping bottom. With the exception of vorticity, which is assumed to have been dissipated by the stratified ambient water column, the same conservation equations used in convective descent but now written for either an oblate spheroid or an ellipsoid are applicable. For the case of collapse on the bottom, a frictional force between the bottom and the collapsing cloud is included which accounts for energy dissipation as a result of the spreading. Other than the changes noted above, the same equations presented in Brandsma and Divoky (1976) apply.

C2.2.3 Transport-diffusion

When the rate of spreading in the dynamic collapse phase becomes less than an estimated rate of spreading due to turbulent diffusion in both the horizontal and vertical directions, the collapse phase is terminated. Laboratory experiments by Johnson et al. (1994) as well as field data collected by Kraus (1991) imply that fine material is lost to the water column at the top of the collapsing cloud. As these

particles leave the main body of material, they are also stored in small clouds that are characterized by a Gaussian distribution, i.e.,

$$C = \frac{m}{(2\pi)^{3/2}\sigma_x\sigma_y\sigma_z} \exp\left\{-\frac{1}{2}\left[\frac{(x-x_o)^2}{\sigma_x^2} + \frac{(y-y_o)^2}{\sigma_y^2} + \frac{(z-z_o)^2}{\sigma_z^2}\right]\right\} \quad (1)$$

where

m = volume of solids in the cloud, ft^3

$\sigma_x,\sigma_y,\sigma_z$ = standard deviations, ft

x,y,z = spatial coordinates, ft

x_o,y_o,z_o = coordinates of cloud centroid, ft

At the end of each time-step, each cloud is advected horizontally by the input velocity field. The new position of the cloud centroid is determined by

$$x_{o_{new}} = x_{o_{old}} + u \cdot \Delta t$$

$$z_{o_{new}} = z_{o_{old}} + w \cdot \Delta t \quad (2)$$

where

u,w = input ambient velocities, fps

Δt = long-term time-step, sec

In addition to the advection or transport of the cloud, the cloud grows both horizontally and vertically as a result of turbulent diffusion. The horizontal diffusion is based upon the commonly assumed four-thirds power law. Therefore, the diffusion coefficient, $K_{x,z}$, (up to a maximum value of 100 ft^2/s) is given as

$$K_{x,z_{new}} = A_L L^{4/3} \quad (3)$$

where A_L is an input dissipation parameter and L is set equal to four standard deviations. As illustrated in Figure 2.4 of Brandsma and Divoky (1976), a value of 100 ft^2/sec for the horizontal diffusion coefficient corresponds to a length scale of 10^3-10^4 feet. With the computational grid cell typically being on the order of 100-500 ft, a length scale greater than 1,000 ft would normally be associated with mean flow rather than turbulence. Thus, restricting the diffusion coefficient to less than 100 ft^2/sec is reasonable.

Horizontal growth is achieved by employing the Fickian expression

$$\sigma_{x,z} = (2K_{x,z}t)^{1/2} \quad (4)$$

where

$\sigma_{x,z}$ = a standard deviation

t = time since formation of the cloud

From Equation 4,

$$\frac{d\sigma_{x,z}}{dt} = K_{x,z}(2K_{x,z}t)^{-1/2} \quad (5)$$

and thus,

$$\sigma_{x,z_{new}} = \sigma_{x,z_{old}} + \frac{K_{x,z_{new}}}{\sigma_{x,z_{old}}} \Delta t \quad (6)$$

where

$\sigma_{x,z^{new}} = \sigma_{x,z}$ at the current time step, Δt

$\sigma_{x,z^{old}} = \sigma_{x,z}$ at the previous time step, Δt

In a similar manner, the vertical growth is written as

$$\sigma_{y_{new}} = \sigma_{y_{old}} + \frac{K_y}{\sigma_{y_{old}}} \Delta t \quad (7)$$

where K_y is a function of the stratification (including the effect of the sediment) of the water column. The maximum value of K_y is input as a model coefficient and occurs when the water density is uniform. It should be noted that since computations are made for each solid fraction independently from the remaining material, the effect of the total volume of suspended material on reducing vertical diffusion is not modeled. This can sometimes lead to confusing results; e.g., a small amount of sand may become diffused over the entire water column while a much larger amount of silt might have its vertical diffusion suppressed due to the larger concentration. Modifications to correct this problem are under investigation.

If long-term output is desired at the end of a particular time-step, the concentration of each solid type is given at each grid point by summing the contributions from individual clouds to yield

$$C_T = (2\pi)^{-3/2} \sum_{i=1}^{N} \frac{m_i}{\sigma_{x_i}\sigma_{y_i}\sigma_{z_i}} \exp\left\{-\frac{1}{2}\left[\frac{(x - x_{o_i})^2}{\sigma_{xi}^2} + \frac{(y - y_{o_i})^2}{\sigma_{y_i}^2} + \frac{(z - z_{o_i})^2}{\sigma_{z_i}^2}\right]\right\} \quad (8)$$

where N is the number of small clouds of a particular solid type and y (the vertical position at which output is desired) is specified through input data. This approach for the transport-diffusion phase follows the work of Brandsma and Sauer (1983). The surface and all solid boundaries except the bottom are handled by assuming reflection from the boundaries.

In addition to the horizontal advection and diffusion of material, settling of the suspended solids also occurs. Therefore, at each net point the amount of solid material deposited on the bottom and a corresponding thickness are also determined. Since a normal distribution is assumed for material in the small clouds, deposited material is also assumed to take a normal distribution horizontally on the bottom. A basic assumption in the model is that once material is deposited on the bottom, it remains there; i.e., no allowance is made for either erosion or bed-load movement of material. However, deposition is prohibited if the computed bottom shear stress exceeds a specified critical shear stress for deposition for each solid fraction. This allows for application at dispersive sites.

The discussion presented above for transport-diffusion of solids also applies to the disposed fluid with its dissolved constituents. It is conservatively assumed that all of the fluid remaining in the collapsing cloud and its dissolved contaminants are released to the water column in Gaussian clouds during collapse. The contaminants are assumed to be conservative with no further adsorption on or desorption from the solids in the water column or deposited on the bottom.

C2.3 Model Capabilities

STFATE enables the computation of the physical fate of dredged material disposed in open water. The following discussion describes particular capabilities or special features.

C2.3.1 Disposal Methods

Disposal is assumed to occur from either a split-hull barge or a hopper dredge.

C2.3.2 Ambient Environment

As illustrated in Figure C-2, time-invariant velocity profiles that allow for flow reversal can be prescribed. These profiles are applied at each grid point. Another option is to specify a time-invariant, spatially varying depth-averaged velocity. The ambient density profile at the deepest point on the grid must also be prescribed.

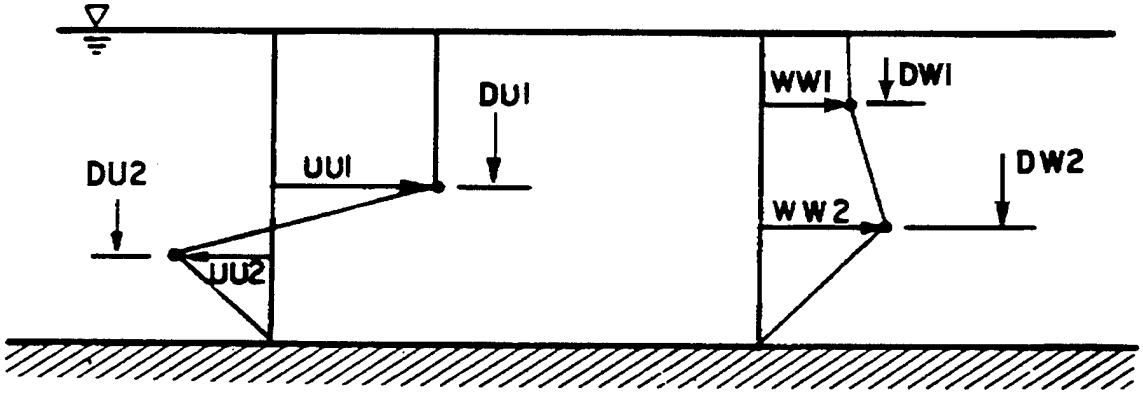


Figure C-2. Velocity Profile Available for Use in PC Model.

C2.3.3 Time-varying Fall Velocities

If a solid fraction is specified as being cohesive, the settling velocity is computed as a function of the suspended sediment concentration of that solid type. The algorithm used is

$$V_s \begin{cases} = 0.000034 \quad \text{if} \quad C \leq 25 \text{ mg/L} \\ = 0.0000225 + 1.6 \times 10^{-7} C^{4/3} \quad \text{if} \quad 25 \leq C \leq 3000 \text{ mg/L} \\ = 0.0069 \quad \text{if} \quad C > 3000 \text{ mg/L} \end{cases} \qquad (9)$$

where

V_s = settling velocity, fps

C = suspended sediment concentration, mg/L

This approach is taken from Ariathurai et al. (1977).

C2.3.4 Conservative Constituent Computations

STFATE allows for the dredged material to contain a conservative constituent with perhaps a nonzero background concentration of that constituent. Computing the resultant time-history of that concentration provides information on the dilution that can be expected over a period of time at the disposal site and enables the computation of mixing zones in water column evaluations.

C2.4 Model Input

Input data for the model are grouped into the following general areas: (1) description of the disposal site, (2) description of site velocities, (3) controls for input, execution, and output, (4) description of the dredged materials, (5) description of the disposal operation, and (6) model coefficients.

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 dissolved contaminants. Each solid fraction has to have a volumetric concentration, a specific gravity, a settling velocity, a void ratio for bottom deposition, critical shear stress, and information on whether or not the fraction is cohesive and/or strippable. For initial-mixing calculations, information on initial concentration, background concentration, and water quality standards for the constituent to be modeled have to be specified. The description of the disposal operation includes the position of the disposal barge or hopper dredge on the grid; the barge or hopper dredge velocity, dimensions, and draft; the volume of dredged material to be discharged. Coefficients are required for the model 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 C-2 lists the necessary input parameters with their corresponding units. Table C-2 also lists the input parameters for determining the contaminant of concern to be modeled based on dilution needs. More detailed descriptions and guidance for selection of values for many of the parameters is provided directly online in the system.

C2.5 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 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.

Table C-2. STFATE Model Input Parameters.

Parameter	Disposal* Operation Types	Units	Options**
Contaminant Selection Data			
Solids concentration of dredged material		g/L	
Contaminant concentration in the bulk sediment		µg/Kg	
Contaminant concentration in the elutriate		µg/L	
Contaminant background concentration at disposal site		µg/L	
Contaminant water quality standards		µg/L	
Site Description			
Number of grid points (left to right)	H, B		
Number of grid points (top to bottom)	H, B		
Spacing between grid points (left to right)	H, B	ft	
Spacing between grid points (top to bottom)	H, B	ft	
Constant water depth	H, B	ft	C
Roughness height at bottom of disposal site	H, B	ft	
Slope of bottom in x-direction	H, B	degrees	
Slope of bottom in z-direction	H, B	degrees	
Number of points in density profile	H, B		
Depth of density profile point	H, B	ft	
Density at profile point	H, B	g/cc	
Salinity of water at disposal site	H, B	ppt	Optional
Temperature of water at disposal site	H, B	Celsius	Optional
Grid points depths	H, B	ft	V
Velocity Data			
Type of velocity profile	H, B		
Water Depth for Averaged Velocity	H, B	ft	
Vertically averaged x-direction velocity	H, B	ft/sec	
Vertically averaged z-direction velocity	H, B	ft/sec	
Water depths for 2-point profile	H, B	ft	
Velocities for 2-point profile in x-direction	H, B	ft/sec	
Velocities for 2-point profile in z-direction	H, B	ft/sec	
Velocities for entire grid in x-direction	H, B	ft/sec	
Velocities for entire grid in z-direction	H, B	ft/sec	
Input, Execution and Output Keys			
Processes to simulate	H, B		
Duration of simulation	H, B	sec	
Long-term time step for diffusion	H, B	sec	
Convective descent output option	H, B		

Table C-2. STFATE Model Input Parameters (continued)

Parameter	Disposal* Operation Types	Units	Option**
Input, Execution and Output Keys (continued)			
Collapse phase output option	H, B		
Number of print times for long-term diffusions	H, B		
Location of upper left corner of mixing zone on grid	H, B	ft	
Location of lower right corner of mixing zone on grid	H, B	ft	
Water quality standards at border of mixing zone for contaminant of concern	H, B	mg/L	
Contaminant of concern	H, B		
Contaminant concentration in sediment	H, B	mg/Kg	
Background concentration at disposal site	H, B	mg/L	
Location of upper left corner of zone of initial dilution (ZID) on grid	H, B	ft	
Location of lower right corner of zone of initial dilution (ZID) on grid	H, B	ft	
Water quality standards at border of ZID for contaminant of concern	H, B	mg/L	
Number of depths in water column for which output is desired	H, B		
Depths for transport - diffusion output	H, B	ft	
Predicted initial concentration in fluid fraction	H, B	mg/L	
Dilution required to meet toxicity standards	H, B	percent	
Dilution required to meet toxicity standards at border of ZID	H, B	percent	
Material Description Data			
Total volume of dredged material in the Hopper dredge	H	yd^3	
Number of distinct solid fractions	H, B		
Solid-fraction descriptions	H, B		
Solid-fraction specific gravity	H, B		
Solid-fraction volumetric concentration	H, B	yd^3/yd^3	
Solid-fraction fall velocity	H, B	ft/sec	
Solid-fraction deposited void ratio	H, B		
Solid-fraction critical shear stress	H, B	lbs/sq ft	
Cohesive? (yes or no)	H, B		
Stripped during descent? (yes or no)	H, B		
Moisture content of dredged material as multiple of liquid limit	H, B		Cohesive
Water density at dredging site	H, B	g/cc	
Salinity of water at dredging site	H, B	ppt	Optional
Temperature of water at dredging site	H, B	Celsius	Optional
Desired number of layers	B		
Volume of each layer	B	yd^3	
Velocity of vessel in x-direction during dumping of each layer	B	ft/sec	
Velocity of vessel in z-direction during dumping of each layer	B	ft/sec	

Table C-2. STFATE Model Input Parameters (continued)

Parameter	Disposal* Operation Types	Units	Option**
Disposal Operation Data			
Location of disposal point from top of grid	H, B	ft	
Location of disposal point from left edge of grid	H, B	ft	
Length of disposal vessel bin	H, B	ft	
Width of disposal vessel bin	H, B	ft	
Distance between bins	H	ft	
Pre-disposal draft of Hopper	H	ft	
Post-disposal draft of Hopper	H	ft	
Time required to empty all Hopper bins	H	sec	
Number of Hopper bins opening simultaneously	H		
Number of discrete openings of sets of Hopper bins	H		
Vessel velocity in x-direction during each opening of a set of Hopper bins	H	ft/sec	
Vessel velocity in z-direction during each opening of a set of Hopper bins	H	ft/sec	
Bottom depression length in x-direction	H, B	ft	Optional
Bottom depression length in z-direction	H, B	ft	Optional
Bottom depression average depth	H, B	ft	Optional
Pre-disposal draft of disposal vessel	B	ft	
Post-disposal draft of disposal vessel	B	ft	
Time needed to empty disposal vessel	B	sec	
Coefficients			
Settling coefficient	H, B		
Apparent mass coefficient	H, B		
Drag coefficient	H, B		
Form drag for collapsing cloud	H, B		
Skin friction for collapsing cloud	H, B		
Drag for an ellipsoidal wedge	H, B		
Drag for a plate	H, B		
Friction between cloud and bottom	H, B		
4/3 Law horizontal diffusion dissipation factor	H, B		
Unstratified water vertical diffusion coefficient	H, B		
Cloud/ambient density gradient ratio	H, B		
Turbulent thermal entrainment	H, B		
Entrainment in collapse	H, B		
Stripping factor	H, B		

* The use of a parameter for disposal operations by a multiple bin hopper dredge is indicated in the table by an H while a parameter used for disposal from a split-hull barge or scow is indicated by a B.

** The use of a parameter for the constant depth option or variable depth option is indicated in the table by a C or V, respectively. Other optional uses for parameters are so indicated.

At the conclusion of the collapse phase, 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. The model performs 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, 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. Similarly, contour plots of concentration can be generated at the requested water depths and at the selected print times.

C2.6 General Instructions for Running the Model

C2.6.1 Target Hardware Environment

The system is designed for the 80386 based processor class of personal computers using DOS. This does not constitute official endorsement or approval of these commercial products. In general, the system requires a math coprocessor, 640 KB of RAM and a hard disk. The STFATE executable model requires about 565 KB of free RAM to run; therefore, it may be necessary to unload network and TSR software prior to execution. The model is 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.

C2.6.2 Installation and Starting

An executable version of the STFATE model can be downloaded from the Internet web site http://www.epa.gov/OST/pubs/ITM.html. Go to "Short Term Fate Model for Open Water Barge and Hopper Discharges (STFATE)," and download the two zipped files "STFATE1.zip" and STFATE2.zip" to a directory on the hard disk dedicated for the STFATE model, e.g. C:\STFATE. Unzip each file using **pkunzip**, type "loadfate" to dearchive the files, then type "STFATE" to start the model. (When unzipping, the user should type "y" when queried as to overwriting a particular file. A demonstration model, "DEMO," can also be downloaded to another directory on the hard disk. Type "startdem" to unzip and run the demonstration model.

C2.6.3 User Interface

The STFATE module of ADDAMS employs 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 clear a single character in a cell. The spacebar will insert a space in alphanumeric cells. The PgDn key advances the cursor to the next data entry screen and the PgUp key returns control to the previous data entry screen. The Esc key returns control to exit to the previous menu without loss of data. 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.

C2.7 Steps in Using the Model

The menu-driven environment for applying the model is illustrated in Figures C-3 and C-4.

The general steps and menus used in applying the model for a disposal operation are as follows:

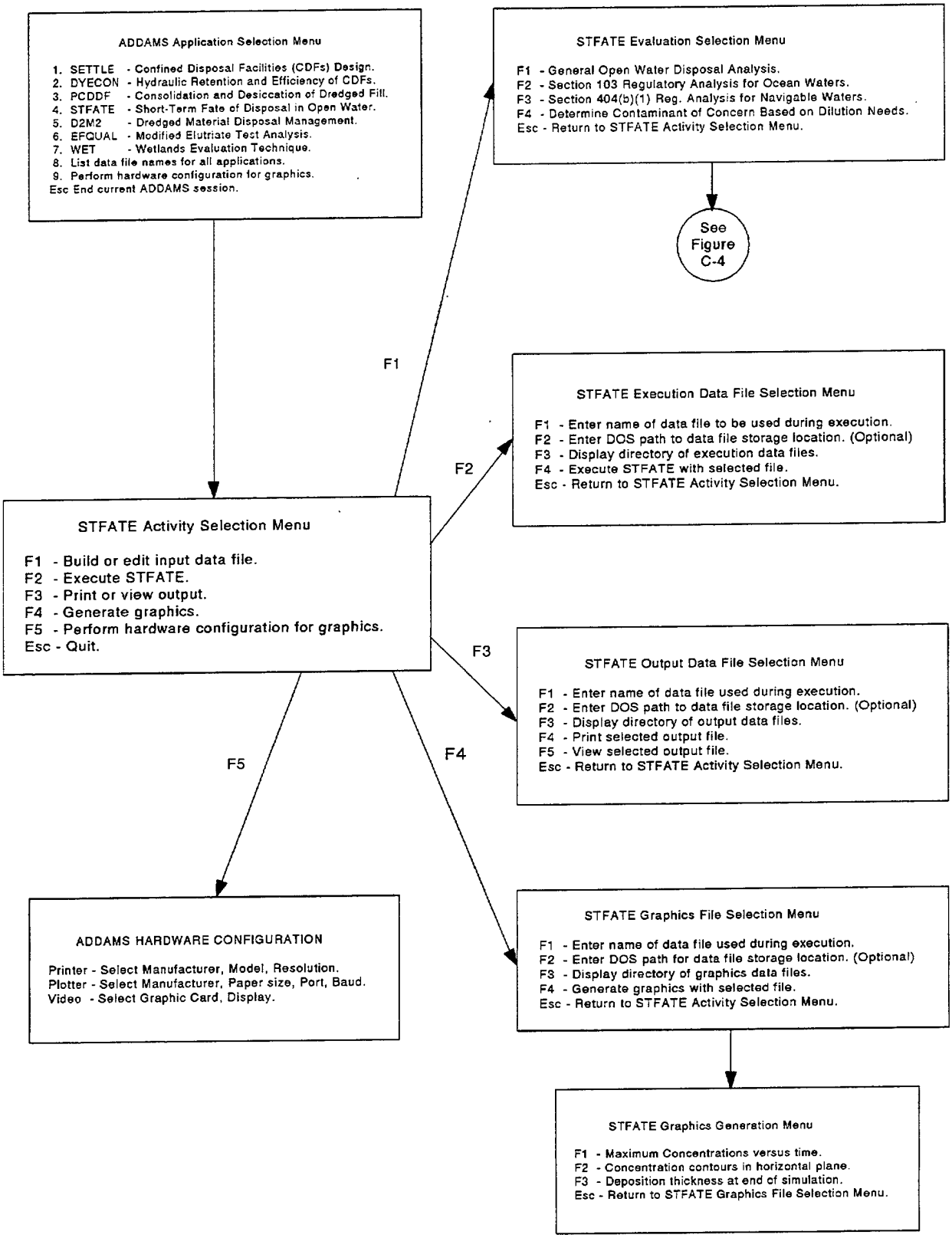


Figure C-3. Menu Tree for STFATE Model.

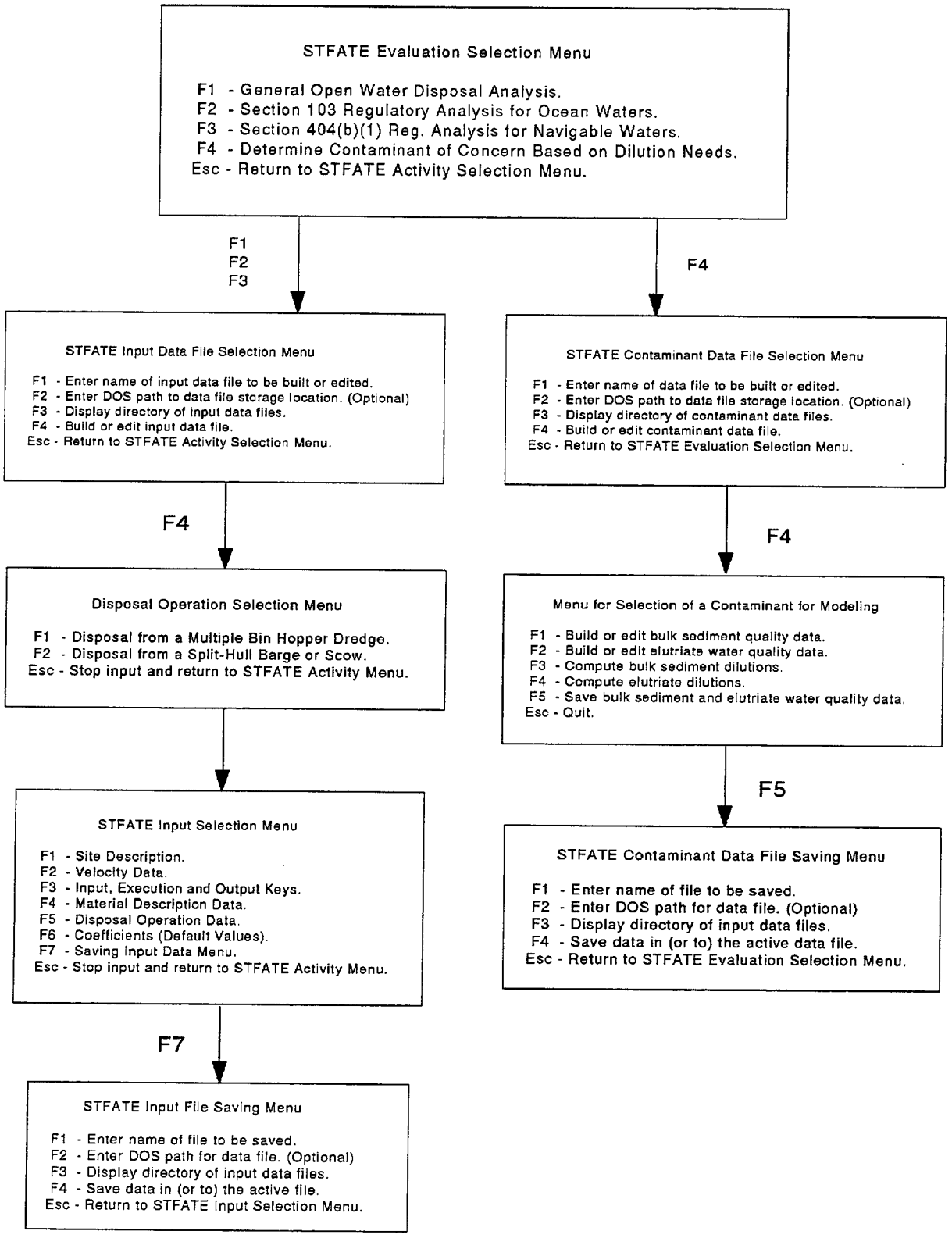


Figure C-4. STFATE Input Menus.

a. Starting

Change the directory to make directory containing the STFATE module the default directory. Start the program by entering ADDAMS or STFATE at the DOS prompt. If started by entering ADDAMS, 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 module provided on diskette with this manual is named STFATE. STFATE consists of programs for evaluating open-water disposal of dredged material. Select the STFATE application module from the Application Selection Menu. The module will display some logos and then a reference screen with points of contact. After the user strikes any key, the module displays the STFATE Activity Selection Menu. If started by entering STFATE, the module starts with STFATE logos and the reference screen and proceeds in the same manner as if the module was started by entering ADDAMS.

b. Activity Selection Menu

The activity selection menu may be considered the main menu for the STFATE application. The first option is used to build or edit an input data file. The second option executes the simulation. The third option is used to print or view output. The fourth option generates graphics. The fifth option is used to configure the graphics software for the hardware present.

c. Evaluation Selection Menu

Selecting F1 from the STFATE Activity Selection Menu brings up the STFATE Evaluation Selection Menu. There are four options available. The two options of interest here are F3 - Section 404(b)(1) Reg. Analysis for Navigable Waters (see step g) and F4 - Determine Contaminant of Concern Based on Dilution Needs (see step d). This option is used only for Tier II evaluations.

d. Contaminant Data File Selection Menu

Selecting F4 from the Evaluation Selection Menu brings up this menu which has the same structure as the Input Data File Selection Menu (see step g). Use one of the options to select an active file.

e. Menu for Selection of a Contaminant for Modeling

Selecting F4 from the Contaminant Data File Selection Menu brings up this 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 standards. Execution of the open-water disposal model for these Tier II analyses allows use of only one contaminant; this option is used to select that contaminant.

Bulk sediment and background contaminant concentrations and water quality standards 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 water quality standards are required to compute the required dilutions for the dissolved contaminants. The contaminant requiring the largest dilution should be subsequently modeled in the Tier II water quality analysis.

f. Contaminant Data File Saving Menu

This menu has the same structure as the Input File Saving Menu (see step j). The contaminant data files are saved with an extension of .DUD. The contaminant data files store the user-specified data, including contaminant names, particulate-associated concentrations of contaminants in the bulk sediment, sediment solids concentration, standard elutriate concentrations of contaminants, water quality standards for the contaminants, and concentrations of contaminants in the background water at the disposal site.

g. Input Data File Selection Menu

Selecting F3 from the Evaluation Selection Menu brings up the Input Data File Selection Menu. An input data file needs to be selected only when the user wants to edit data that were previously entered. The changes can be saved to the same file or to a new file. The first option is used to specify the name of the file to be used. 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. If a path is not specified, the program will use the default directory, where the STFATE program is, for file storage. The third option displays a directory of STFATE input data files for the current path, that is files having an extension of .DUI in the directory specified in the path. An existing data file name may be selected from the list to use as the active data file name for reading existing data. After the

input file has been selected, press F4 to build or edit the input data file. The input data that are stored in the selected file are then read and will later be displayed on the input data screens to be reviewed and edited. If the specified file could not be found (did not exist), the program will provide the user the opportunity to initialize the file and start creating a new data set.

h. Disposal Operation Selection Menu

Selecting F4 from the Input Data File Selection Menu brings up this 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: F1 - Disposal from a Multiple Bin Hopper Dredge, or F2 - Disposal from a Split-Hull Barge or Scow. The STFATE Input Selection Menu will then be displayed.

i. Input Selection Menu

Five types of input data have to be entered as shown in Table C-2 and Figure C-4, 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. Enter data by paging down through the data entry screens, making selections and filling in the cells for each option. An input data file may be written at any point to save all the data that have been entered up to that point. After entering all of the data, the data must be saved before returning to the STFATE Activity Selection Menu to avoid losing the changes. The data are saved in input data and execution data files by selecting F7 from the Input Selection Menu to bring up the Input File Saving Menu.

j. Input File Saving Menu

This menu provides the opportunity to write an input data file to save the input data for future editing under the STFATE Input Selection Menu and an execution data file for use during execution of the STFATE model. 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 operation and contain data only for the specific options selected in the input. The files are stored with the same name as the input data file but with an extension of .DUE instead of .DUI. The input data file stores data for all possible options in evaluations, disposal operations and methods of data entry, allowing the user to perform comparisons between options without re-entering

previously specified data. This menu is similar to the Input Data Selection Menu (see step g). The only difference is that the name of the file to be saved instead of read should be specified. The same file as read can be used but the input and execution data files will be overwritten. If the input data are complete, an execution data file will also be saved. After the files are saved, the program returns control to the Input Selection Menu. At this point data entry is complete and the user should return to the Activity Selection Menu by hitting the Esc key.

k. Execute

Selecting F2 from the Activity Selection Menu (see step b) initiates execution by bringing up the Execution Data File Selection Menu. After selecting the execution data file (same procedure as the other file selection menus), pressing F4 begins the simulation. This option uses the execution data file to generate an output file and three graphics files of the same name as the execution data file selected but with an extension of .DUO, .DUP, .DUC, and .DUT, respectively, instead of .DUE. The execution may take a few minutes or several hours, depending on the simulation selected and the computer hardware used, but typically 30 minutes is sufficient. After termination of the simulation the program returns to the Activity Selection Menu.

l. Print or View Output

Select F3 from the Activity Selection Menu to print or view text output. A STFATE Output Data File Selection Menu will be displayed that is similar to the other file selection menus. The output files have the same name as the execution data files used to generate them except that they have a .DUO extension instead of a .DUE extension. The other difference in the menu is that it has an option to view the output on the monitor using the LIST.COM utility program. Instructions on using the LIST program are provided on the menu bar and on-line by pressing the ? key. The output is an ASCII text file having 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 STFATE program, using the DOS print command or a word processor. The output contains an interpretive listing of the input data, computational indicators, convective descent results, collapse results, information on cloud generation for transport-diffusion simulation, accumulation and thickness of deposited materials, spatial distribution of concentrations of materials in the water column, and water quality comparisons with standards for determining water quality violations or mixing zone requirements.

m. Generate Graphics

Selecting F4 from the Activity Selection Menu brings up the Graphics File Selection Menu which is similar to the other file selection menus. Unlike the other file selection procedures, there are three graphics files, not one as for input, execution and output. All three files have the same name as the execution file used to generate the output and graphics files, but have different extensions. The graphics file selection procedure uses the graphics file with an extension of .DUT for its directory listing and file searching. Selecting F4 (Generate Graphics with Selected File) brings up the Graphics Generation Menu from which there are three options for plotting the data, one for each of three types of graphics files. The three options are F1 - Maximum Concentrations Versus Time, using the file with a .DUP extension; F2 - Concentration Contours in Horizontal Plane, using the file with a .DUC extension; and F3 - Deposition Thickness at End of Simulation, using the file with a .DUT extension. The plots can be viewed on the monitor, or sent to a printer or plotter as desired. However, before plots can be generated the software must be configured for the hardware present.

n. Perform Hardware Configuration for Graphics

Select F5 from the Activity Selection Menu to choose the proper printer, plotter and video information for the computer system being used.

o. 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 which will stop the execution after the next screen update and provide partial output. Alternatively, the program can be stopped by turning off or rebooting the computer, but loss of data and output will occur. These methods of ending are not recommended. Similar methods are available during printing of output.

C2.8 STFATE Application Examples

Two example applications of the use of the numerical model STFATE are described. The first example addresses the instantaneous disposal of dredged material from a split-hull barge or scow. These barges or scows may hold anywhere from approximately 400 to 6000 yd^3 of material and

dispose of the material by means of opening the split-hull and discharging the material through the bottom opening. The material then descends through the water column to the bottom of the water body. The second example illustrates the modeling of dredged material disposal from a multiple-bin hopper dredge. A hopper dredge fills its bins with dredged material and then transports it to the disposal site where it discharges the material. Each bin has a separate opening in the ship's bottom through which the dredged material is discharged into the water column. Typically there are anywhere from about 4 to 20 bins in a hopper dredge which can carry a total of approximately 1000 to 9000 yd^3 of material. During disposal one or more bins are opened sequentially until all of the bins have been emptied. The required input data for both examples are described and the results or output from the STFATE model are illustrated and discussed. Additionally, the input and output files for each of the examples are included on an executable version of the STFATE model which can be downloaded from the Internet web site http://www.epa.fov/OST/pubs/ITM.html (see section C2.6.2)

C2.8.1 Split-hull Barge or Scow Example

An example of dredged material disposal is modeled for an instantaneous disposal using STFATE for a 3000 yd^3 disposal from a split-hull barge at a constant 40 ft depth site for Section 404(b)(1) regulatory analysis for water quality. The input data for this example are given in Table C-3. No mixing zone dimensions are specified for this example, therefore the dimensions of a mixing zone required to meet the water quality standard are calculated. A description follows for entering the required example data and the use of the STFATE module.

C2.8.1.1 Entering STFATE and the Input Data File Selection Menu

The STFATE model is executed from the disk operating system (DOS) prompt and the "STFATE Activity Selection Menu" is reached as presented earlier. The menus are shown in Figs. C-3 and C-4. To proceed, the "Build or edit input data file" option is selected and the "STFATE - Short-term Fate of a Disposal in Open Water Evaluation Selection Menu" appears. For this example, the option "Section 404(b)(1) Reg. Analysis for Navigable Waters" is selected. Next, the "STFATE Input Data File Selection Menu" is presented and the key F1 is pressed to enter name of input data file to be built or edited. For this example, BARGE is typed and the ENTER key is pressed. Option F4, "Build or edit input data file," is then selected to read the input data file if it exists or to initialize it if it is a new file. A descriptive title, "Barge dump without specified mixing zone (Tier II W.Q.)" is typed and entered (Press ENTER). The "Disposal Operation Selection Menu" is presented next. "Disposal from a Split-Hull Barge or Scow" is selected and the "STFATE Input Selection Menu"

Table C-3. STFATE Input Variables for Section 404(b)(1) Regulatory Analysis of Navigable Waters Using a Scow/Barge Disposal.

INPUT PARAMETER	UNITS	INPUT VALUE
SITE DESCRIPTION		
Number of grid points (L-R, +z dir)		32
Number of grid points (T-B, +x dir)		32
Grid spacing (L-R), f(V)	ft	50
Grid spacing (T-B), f(V)	ft	200
Constant water depth	ft	40
Bottom roughness	ft	0.005
Bottom slope (x-dir)	deg	0
Bottom slope (z-dir)	deg	0
Number of points in density profile		2
Density at point one (surface)	g/cc	1.0000
Density at point two (bottom)	g/cc	1.0002
VELOCITY		
Type of velocity profile		2 pt
Water depth 2-point profile	ft	30, 38
Vel for 2-point x-direction	ft/s	0.5, 0.3
Vel for 2-point z-direction	ft/s	0, 0
INPUT, EXECUTION & OUTPUT KEYS		
Process to simulate		Disp. from Split-Hull Barge/Scow
Duration of simulation	s	3600
Time step for diffusion, f(V)	s	300
Convective descent output		Yes
Collapse phase output option		Yes
Number of print times for diffusion		Quarterly
Upper left corner mixing zone	ft	0, 0
Lower right corner mixing zone	ft	0, 0
Contaminant	Lead	WQ standard at edge of mixing zone
mg/l	0.0032	
Predicted initial concentration in fluid	mg/L	0.174
Background concentration	mg/L	0.0002
Number of depths for output		2
Depths for output	ft	15, 39

(Continued)

Table C-3 (continued). STFATE Input Variables for Section 404(b)(1) Regulatory Analysis of Navigable Waters Using a Scow/Barge Disposal.

INPUT PARAMETER	UNITS	INPUT VALUE
MATERIAL DESCRIPTION		
Number of solids fraction		3
Solid fraction descriptions		clumps, sand, clay
Solid fraction specific gravity		1.6, 2.7, 2.65
Solid fraction volume concentration	yd^3/yd^3	0.1, 0.2, 0.05 / 0.0, 0.15, 0.10
Solid fraction fall velocity	ft/s	3.0, 0.1, 0.002
Solid fraction depositional void ratio		0.4, 0.6, 5.0
Solid fraction critical shear stress	lb/ft^2	99, 0.025, 0.002
Cohesive (Y/N)		N, N, Y
Stripped during descent(Y/N)		N, Y, Y
Dredge site water density	g/cc	1.0
Number of layers		2
Volume of each layer	yd^3	2000 / 1000
Vessel velocity in x-direction	ft/s	6 / 6
Vessel velocity in z-direction	ft/s	0 / 0
DISPOSAL OPERATION		
Disposal point top of grid	ft	1300
Disposal point left edge of grid	ft	750
Length of vessel bin	ft	200
Width of vessel bin	ft	50
Bottom depression length x-direction	ft	0
Bottom depression length z-direction	ft	0
Bottom depression average depth	ft	0
Predisposal draft	ft	17
Postdisposal draft	ft	5
Time to empty vessel	s	20
COEFFICIENTS		
Settling coef (BETA)		0.0
Apparent mass coefficient (CM)		1.0
Drag coefficient (CD)		0.5
Form drag collapse cloud (CDRAG)		1.0
Skin friction collapse cloud (CFRIC)		0.01
Drag ellipse wedge (CD3)		0.1
Drag plate (CD4)		1.0
Friction between cloud and bottom (FRICTN)		0.01
4/3 Law horizontal diffusion coefficient (ALAMDA)		0.001
Unstratified vertical diffusion coefficient (AKY0)		0.025
Cloud/ambient density gradient ratio (GAMA)		0.25
Turbulent thermal entrainment (ALPHA0)		0.235
Entrainment collapse (ALPHAC)		0.1
Stripping factor (CSTRIP)		0.003

(Concluded)

appears on the computer monitor. Now the entering of the input given in Table C-3, developed from Table C-2, begins.

C2.8.1.1.1 Site Description Data

"F1 - Site Description" is selected from the "STFATE Input Selection Menu" by pressing key F1 or by using arrow keys to highlight the selection and pressing ENTER. The number of grid points is selected as 32 in both the x-direction (top to bottom) and z-direction (left to right). The spacing is picked as 50 ft in the z-direction and 150 ft in the x-direction. These spacings are selected since the velocity, described later, is 0.5 ft/s in the x-direction and 0.0 ft/s in the z-direction. Thus, the disposal site (Fig. C-5) is 1550 ft wide and 4650 ft long. In the first screen of data entry, the constant water depth is entered as 40 ft and the bottom roughness is input as the mid range value of 0.005. The bottom is assumed to be flat so a slope of zero is entered for the x- and z-directions. Data entry on this screen is complete so the "PAGE DOWN" key is pressed. The next screen requests information describing the water density profile at the site. First, the number of points required to describe the density profile is entered as 2. This is the minimum number because the surface (zero depth) and the bottom (40 ft) must be entered. Additional depths may be needed to describe more complicated profiles with the maximum number of depths being 5. Next, the method of entering density data is determined by selecting "YES" for direct entry of depth and density or "NO" for entering depth, salinity and temperature from which density is automatically computed. For this example, "YES" is selected and highlighted boxes are presented for entering depth (ft) and density (g/cm^3) of 0.0, 1.0000 and 40.0, 1.0002 respectively. The data entry is now complete and pressing PAGE DOWN results in the return of the "STFATE Input Selection Menu".

C2.8.1.1.2 Velocity Data

The selection of "F2-Velocity Data" from the "STFATE Input Selection Menu" brings the "Velocity Profile Selection Menu" up on the monitor. For this example, a 2-point velocity profile (Fig. C-6) for a constant depth is selected by pressing the F2 key or highlighting the selection using arrow keys and pressing PAGE DOWN or ENTER. A velocity profile data entry screen appears and the velocity and depth data are entered by typing the data in the highlighted box and then pressing ENTER. A x-direction velocity of 0.5 ft/s at a depth of 30 ft and 0.3 ft/s at 38 ft are entered. The z-direction velocity is 0 ft/s. Although zero velocity can be input, it is recommended that the speed of the resultant velocity vector be set at least 0.1 ft/s because most open bodies of water have some motion occurring at all times. When the input of velocity data is complete, press PAGE DOWN to return to the "STFATE Input Selection Menu."

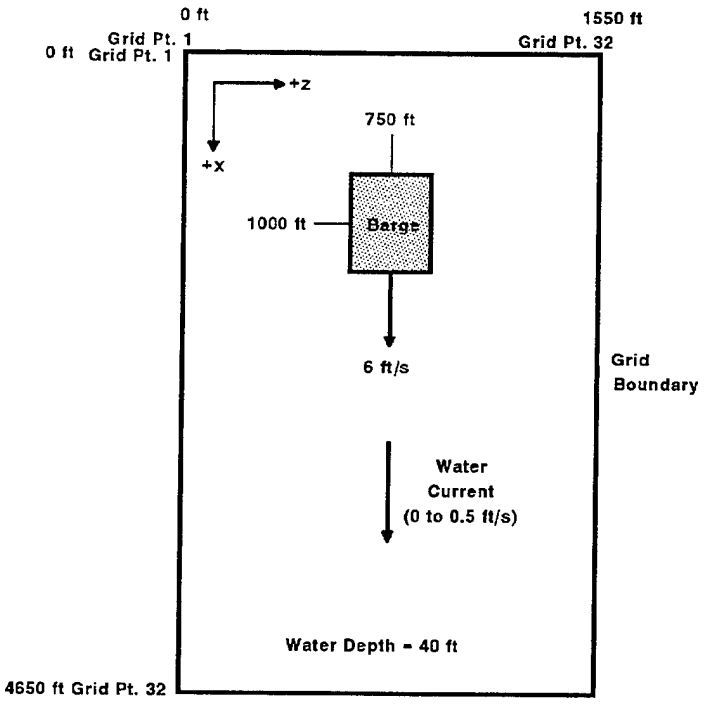


Figure C-5. Schematic of Example Disposal Site for Barge Disposal.

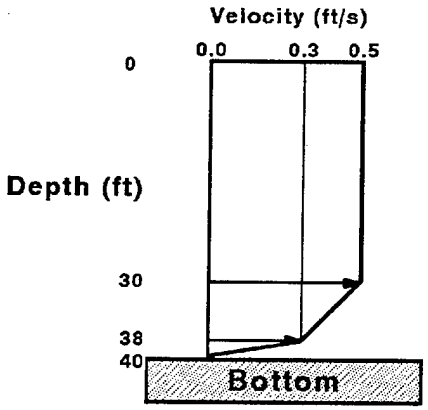


Figure C-6. X-direction Velocity Profile for Barge Example.

C2.8.1.1.3 Input, Execution and Output Keys

At this point, "F3-Input, Execution and Output Keys" is selected in the same method as previously described which brings the "Simulation Selection Menu" to the monitor. Since initial mixing calculations are desired, "F3-DESCENT, COLLAPSE AND LONG-TERM DIFFUSION" is selected. Next, the "Evaluation Selection Menu"appears on the monitor, and since this example requires comparison to water quality concentrations for lead, the "F2-TIER II, COMPARE WATER QUALITY" is chosen. This selection also provides for calculation of the size (length and width) of the mixing zone required to prevent violation of a specified water quality standard. When calculation of the mixing zone is desired, zeros are entered in the boxes for the upper and lower mixing zone corners. In this example, the contaminant of concern is lead which has a background concentration of 0.0002 mg/L. The predicted initial concentration in the fluid fraction is 0.174 mg/L and the water quality standard at border or mixing zone is 0.0032 mg/L. After data are entered, press PAGE DOWN to receive a screen which asks if a zone of initial dilution is desired. For this example, the answer NO is selected using arrow keys. Press PAGE DOWN to specify the depths at which water quality results are desired. The number of depths, between 2 and 5, and the corresponding depth value are entered. In this example, two depths of 15 and 39 ft are entered. Using PAGE DOWN, a data entry screen for the duration of simulation, long term time step, and specifications for printed output are displayed. Since the example is for water column evaluation, a one hour (3600 s) duration and a time step of 300 s is input. Output concerning convective descent and collapse phase are requested to permit review of the simulation. Particular times are not of importance for an example, so NO is chosen and the output is produced quarterly (900, 1800, 2700 and 3600 s). Specific times can be requested by choosing YES and another screen will appear for entering these times, but the times must be increments of the selected time step. Again, PAGE DOWN is pressed returning to the "STFATE Input Selection Menu."

C2.8.1.1.4 Material Description Data

The material description data are entered after choosing the "F4-Material Description Data" from the "STFATE Input Selection Menu." Next, the first material description data entry screen appears and requests information on the number of layers (1 to 6) of material in the barge and the total volume of each layer. In the example, the number of layers is 2 and their volumes are 2000 and 1000 yd^3, respectively. Press PAGE DOWN to get the next screen which provides for specifying the barge velocity in terms of x- and z-direction components for each layer. The barge velocity is assumed to be constant at 6 ft/s in the x-direction. After pressing PAGE DOWN, material separation in the barge is selected as YES for this example; that is, the concentration of solids vary from layer to layer in the discharges from the barge. Also requested is the number of solid fractions (1 to 4) in the

material, such as clumps, gravel, sand, silt or clay; this example uses 3 solid fractions. The next screen inputs the physical characteristics of the solids fractions which are entered in the highlighted boxes on the screen. Typical values and their ranges are shown at the top of the screen. For the example, the input values are shown in Table C-3. Press PAGE DOWN to get the next screen which asks if the adjustment of the entrainment and drag coefficients based on the moisture content is desired. Typically, this is not necessary and NO is selected as was done for the example. The final screen for material description requests input on the density of the dredging site water which is in the barge with the dredged material solids. For the example, the density is entered directly (YES to first question) and the value of 1.000 g/cc is accepted. If the density is different, it can be entered in the highlighted box. If salinity and temperature data only are available, then NO is selected and another screen will appear to allow for the input of those data. At this point, PAGE DOWN is pressed which brings back the "STFATE Input Selection Menu."

C2.8.1.1.5 Disposal Operation Data

To describe the disposal operation, "F5-Disposal Operation Data" is selected which brings up a screen requesting input concerning location of disposal point, length and width of barge bin, the barge draft before and after disposal, and the time needed to empty the barge. The actual data are entered into the respective highlighted boxes on the screen. For this example the location of the disposal point in distance from the top of grid of 1000 ft and from the left edge of grid of 750 ft are entered. The length and width of barge bin are entered as 200 and 50 ft, respectively. The pre- and post-disposal draft are entered as 17 ft and 5 ft, and the time to empty barge is 20 s. Pressing PAGE DOWN gets the next screen which requests information concerning disposal in a pre-existing depression. For the example no depression is used and the values of zero are accepted. PAGE DOWN is pressed again completing data entry and returning to the "STFATE Input Selection Menu."

C2.8.1.1.6 Coefficients

The "STFATE Input Selection Menu" is now displayed on the monitor and the next selection is "F6-Coefficients (Default Values)". Highlighting this selection and pressing ENTER or pressing F6 shows the numerical model coefficients. In most cases in the absence of calibration data, the default values should be chosen, and this is done in the example by pressing PAGE DOWN. If other values are required, then enter them in the highlighted box before pressing PAGE DOWN. Expert guidance should be obtained before using coefficient values other than the default numbers.

C2.8.1.1.7 Saving Input Data Menu

Data entry is now complete as indicated by asterisks by each data entry option. The next step is to save the input data file for use in the execution of STFATE. To proceed, press the F7 key or select "F7-Saving Input Data Menu" from the "STFATE Input Selection Menu" and press ENTER. The "Saving Input Data Menu" appears and requests input as to whether a new file name is desired or the active data file should be used for storing the data. The file name entered at the beginning of the input process appears as the active data file. To save the data or changes in the active data file, select option "F4-Save data in (or to) the active data file". For this example, the active data file BARGE is selected. If the active data file exists, the program indicates the active data file already exists and requests to overwrite the file. Therefore, "Y" is entered to overwrite and the program then requests the entering or editing of a descriptive title for the file. For this example the title "Barge dump without specified mixing zone (Tier II W.Q.)" is entered. Sometimes a file is being edited but the original data file needs to be kept unchanged. At this point a new file name can be selected using "F1-Enter name of file to be saved"; the changes are then saved using option F4. After the data are saved, the "STFATE Input Selection Menu" reappears and all of the selections show an asterisk indicating each selection has been completed. The final step of the input process is to press ESC and return to the "SFTATE Activity Menu" which is the screen at which the input process began. Once the input file is complete and saved, the STFATE model can be executed by first selecting "F2-Execute STFATE" which then requests the input data file to be input. BARGE is input to obtain results for this example which are discussed in the next section.

C2.8.1.2 Description of Barge Disposal Example Output

A general description of the output available from the STFATE has been described. The objective in this section is to illustrate and describe selected portions of the results. The results concerning the accumulation of sediment on the bottom are not discussed since the emphasis is on results for water column concentrations for Tier II and III evaluations. However, bottom sediment accumulation output is contained in the BARGE.DUO file in the STFATE model. Output related to the convective descent and collapse phase is also contained in the output file for the barge example (BARGE.DUO) for information purposes. The results discussed are water column concentrations of the solids fractions, contaminant and the fluid associated with the dredged material in the barge.

Results are in the form of tabular output and graphical presentations which can be accessed through the "STFATE Activity Selection Menu". This screen provides two options for output, "F3-Print or view output" and "F4-Generate graphics." If option F3 is selected, the "STFATE Output Data File Selection Menu" appears, and it provides several possibilities. First, the option "F1-Enter name of

data file used during execution" is used and for this example, the filename BARGE is entered after selecting this option. Once the filename is identified, the options "F4-Print selected output file" or "F5-View selected output file" are used. There is considerable model output and all of the output is usually not desired. The F5 option provides viewing of the output file by paging through it using the PAGE UP and PAGE DOWN keys. The viewing software can also be used to select specific portions of the output for printing selected hard copy. Pressing the ESC key returns the "STFATE Activity Selection Menu".

C2.8.1.2.1 Barge Disposal Water Column Concentrations and Area Distribution

In this example, water column concentrations of the solids fraction and the contaminant were requested at 15 and 39 ft. Thus, the concentrations for clumps, sand, clay and lead at every grid point location for both depths are contained in the output file. In addition, results are available showing the maximum concentration occurring at each grid point over the depth of the water column for the duration of the simulation. The top section of Figure C-7 shows the output for lead concentration in mg/L at 15 ft at the end of the model run (3600 s). The concentration values at each grid point are must be multiplied by the appropriate scale factor, shown at top of the table, to get actual concentration. In this example the maximum value on the grid is 1.6 which is multiplied by 0.001 yielding a maximum lead concentration of 0.0016 mg/L at x- and z-grid locations of 20 and 16, respectively. Recall from the input that the distance between x-grid points is 150 ft and between z-grid points is 50 ft. Therefore, the maximum concentration occurs 2850 ft from top of grid and 750 ft from left edge of grid. The disposal began at an x- and z-distance of 1000 ft (grid point 8) and 750 ft (grid point 16). Both the barge and water velocity were in the positive x-direction so it is reasonable to find the maximum concentration down grid from the disposal point. The lead concentration area at 15 ft is evaluated by determining the number of grid rectangles which have a value representing lead concentration and multiplying it by the area of the grid rectangle (50 x 1500 = 7500 ft^2). A total of 129 grid points have a lead concentration above background of at least 10^{-5} mg/L, an area of 9.675 x 10^5 ft^2. Since the barge and the water current are moving in the positive x-direction, it is expected that the distance in the x-direction should be longer than that in the z-direction. The display of this area in Figure C-7 appears to be wider than it is long, but that is because of the grid spacing. It is actually 1000 ft wide and 1200 ft long (x-direction).

C2.8.1.2.2 Barge Disposal Water Column Concentrations

The water column concentrations over the duration of the simulation are tabulated in the middle sections of Figure C-7. This shows the clay and lead concentrations. The clumps and sand settled to

CONCENTRATIONS ABOVE BACKGROUND OF LEAD (MG/L) IN THE CLOUD 3600.00 SECONDS AFTER DUMP

15.00 FT BELOW THE WATER SURFACE

...MULTIPLY DISPLAYED VALUES BY 0.1000E-02 (LEGEND... + = .LT. .01 . = .LT. .0001 0 = .LT. .000001)

M N= 2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
2	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
14	0	0	0	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	0	0	0	0	0	o
15	0	0	.	.	.	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.	0	0	0	0	0	0	o
16	0	0	+	+	+	+	+	+	+	+	+	.01	.01	.01	.01	.01	+	+	+	+	+	+	0	0	0	0	0	0	o
17	0	0	+	+	+	.01	.01	.02	.04	.06	.08	.10	.11	.12	.11	.10	.08	.06	.04	.02	.01	.01	0	0	0	0	0	0	o
18	0	0	+	.01	.02	.04	.08	.13	.21	.30	.40	.49	.55	.57	.55	.49	.40	.30	.21	.13	.08	.04	0	0	0	0	0	0	o
19	0	0	.01	.02	.05	.10	.19	.33	.51	.73	.97	1.1	1.3	1.4	1.3	1.1	.97	.73	.51	.33	.19	.10	0	0	0	0	0	0	o
20	0	0	.01	.03	.06	.12	.23	.39	.61	.88	1.1	1.4	1.6	1.6	1.6	1.4	1.1	.88	.61	.39	.23	.12	0	0	0	0	0	0	o
21	0	0	+	.01	.03	.07	.13	.23	.36	.51	.68	.84	.95	.99	.95	.84	.68	.51	.36	.23	.13	.07	0	0	0	0	0	0	o
22	0	0	+	+	.01	.02	.03	.06	.10	.14	.19	.23	.26	.28	.26	.23	.19	.14	.10	.06	.03	.02	0	0	0	0	0	0	o
23	0	0	+	+	+	+	+	+	.01	.01	.02	.03	.03	.03	.03	.03	.02	.01	.01	+	+	+	0	0	0	0	0	0	o
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	o
31	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o

SUMMARY OF CONCENTRATIONS FOR CLAY

TIME (HR)	DEPTH (FT)	MAX CONC ABOVE BACKGROUND ON ENTIRE GRID (MG/L)	X-LOC (FT)	Z-LOC (FT)
0.25	15.0	0.306E+03	1500.	750.
0.50	15.0	0.473E+03	1950.	750.
0.75	15.0	0.318E+03	2400.	750.
1.00	15.0	0.293E+03	2400.	750.
0.25	39.0	0.430E+04	1350.	750.
0.50	39.0	0.815E+03	1650.	750.
0.75	39.0	0.427E+03	1950.	750.
1.00	39.0	0.544E+02	2400.	750.

SUMMARY OF CONCENTRATIONS FOR LEAD

TIME (HR)	DEPTH (FT)	MAX CONC ABOVE BACKGROUND ON ENTIRE GRID (MG/L)	MAX CONC ON GRID (MG/L)	X-LOC (FT)	Z-LOC (FT)
0.08	15.0	0.286E-12	0.200E-03	1200.	750.
0.17	15.0	0.212E-02	0.232E-02	1350.	750.
0.25	15.0	0.604E-02	0.624E-02	1500.	750.
0.33	15.0	0.511E-02	0.531E-02	1650.	750.
0.42	15.0	0.435E-02	0.455E-02	1800.	750.
0.50	15.0	0.374E-02	0.394E-02	1950.	750.
0.58	15.0	0.323E-02	0.343E-02	2100.	750.
0.67	15.0	0.281E-02	0.301E-02	2250.	750.

Figure C-7. Selected Output for Barge Disposal.

0.75	15.0	0.246E-02	0.266E-02	2400.	750.
0.83	15.0	0.216E-02	0.236E-02	2550.	750.
0.92	15.0	0.191E-02	0.211E-02	2700.	750.
1.00	15.0	0.169E-02	0.189E-02	2850.	750.
0.08	39.0	0.103E-01	0.105E-01	1200.	750.
0.17	39.0	0.231E-02	0.251E-02	1350.	750.
0.25	39.0	0.112E-02	0.132E-02	1500.	750.
0.33	39.0	0.950E-03	0.115E-02	1650.	750.
0.42	39.0	0.810E-03	0.101E-02	1800.	750.
0.50	39.0	0.695E-03	0.895E-03	1950.	750.
0.58	39.0	0.601E-03	0.801E-03	2100.	750.
0.67	39.0	0.522E-03	0.722E-03	2250.	750.
0.75	39.0	0.457E-03	0.657E-03	2400.	750.
0.83	39.0	0.401E-03	0.601E-03	2550.	750.
0.92	39.0	0.355E-03	0.555E-03	2700.	750.
1.00	39.0	0.315E-03	0.515E-03	2850.	750.
0.08	0.0	0.524E-02	0.544E-02	1200.	750.
0.17	0.0	0.135E-02	0.155E-02	1350.	750.
0.25	0.0	0.185E-02	0.205E-02	1500.	750.
0.33	0.0	0.156E-02	0.176E-02	1650.	750.
0.42	0.0	0.133E-02	0.153E-02	1800.	750.
0.50	0.0	0.114E-02	0.134E-02	1950.	750.
0.58	0.0	0.988E-03	0.119E-02	2100.	750.
0.67	0.0	0.859E-03	0.106E-02	2250.	750.
0.75	0.0	0.752E-03	0.952E-03	2400.	750.
0.83	0.0	0.661E-03	0.861E-03	2550.	750.
0.92	0.0	0.584E-03	0.784E-03	2700.	750.
1.00	0.0	0.518E-03	0.718E-03	2850.	750.
0.08	10.0	0.237E-01	0.239E-01	1200.	750.
0.17	10.0	0.695E-02	0.715E-02	1350.	750.
0.25	10.0	0.421E-02	0.441E-02	1500.	750.
0.33	10.0	0.357E-02	0.377E-02	1650.	750.
0.42	10.0	0.304E-02	0.324E-02	1800.	750.
0.50	10.0	0.261E-02	0.281E-02	1950.	750.
0.58	10.0	0.226E-02	0.246E-02	2100.	750.
0.67	10.0	0.196E-02	0.216E-02	2250.	750.
0.75	10.0	0.171E-02	0.191E-02	2400.	750.
0.83	10.0	0.151E-02	0.171E-02	2550.	750.
0.92	10.0	0.133E-02	0.153E-02	2700.	750.
1.00	10.0	0.118E-02	0.138E-02	2850.	750.
0.08	20.0	0.393E-01	0.395E-01	1200.	750.
0.17	20.0	0.126E-01	0.128E-01	1350.	750.
0.25	20.0	0.683E-02	0.703E-02	1500.	750.
0.33	20.0	0.578E-02	0.598E-02	1650.	750.
0.42	20.0	0.492E-02	0.512E-02	1800.	750.
0.50	20.0	0.423E-02	0.443E-02	1950.	750.
0.58	20.0	0.365E-02	0.385E-02	2100.	750.
0.67	20.0	0.318E-02	0.338E-02	2250.	750.
0.75	20.0	0.278E-02	0.298E-02	2400.	750.
0.83	20.0	0.244E-02	0.264E-02	2550.	750.
0.92	20.0	0.216E-02	0.236E-02	2700.	750.
1.00	20.0	0.191E-02	0.211E-02	2850.	750.

Figure C-7. Selected Output for Barge Disposal. (continued)

0.08	30.0	0.239E-01	0.241E-01	1200.	750.
0.17	30.0	0.794E-02	0.814E-02	1350.	750.
0.25	30.0	0.414E-02	0.434E-02	1500.	750.
0.33	30.0	0.350E-02	0.370E-02	1650.	750.
0.42	30.0	0.299E-02	0.319E-02	1800.	750.
0.50	30.0	0.256E-02	0.276E-02	1950.	750.
0.58	30.0	0.221E-02	0.241E-02	2100.	750.
0.67	30.0	0.193E-02	0.213E-02	2250.	750.
0.75	30.0	0.168E-02	0.188E-02	2400.	750.
0.83	30.0	0.148E-02	0.168E-02	2550.	750.
0.92	30.0	0.131E-02	0.151E-02	2700.	750.
1.00	30.0	0.116E-02	0.136E-02	2850.	750.
0.08	40.0	0.531E-02	0.551E-02	1200.	750.
0.17	40.0	0.172E-02	0.192E-02	1350.	750.
0.25	40.0	0.924E-03	0.112E-02	1500.	750.
0.33	40.0	0.781E-03	0.981E-03	1650.	750.
0.42	40.0	0.666E-03	0.866E-03	1800.	750.
0.50	40.0	0.572E-03	0.772E-03	1950.	750.
0.58	40.0	0.494E-03	0.694E-03	2100.	750.
0.67	40.0	0.430E-03	0.630E-03	2250.	750.
0.75	40.0	0.376E-03	0.576E-03	2400.	750.
0.83	40.0	0.330E-03	0.530E-03	2550.	750.
0.92	40.0	0.292E-03	0.492E-03	2700.	750.
1.00	40.0	0.259E-03	0.459E-03	2850.	750.

ESTIMATES OF AREAS CURRENTLY IN VIOLATION (SNAPSHOT) AND MIXING ZONES (ACCUMULATED AREA OF VIOLATION)

TIME	SNAPSHOT			ACCUMULATED		
(SEC)	AREA(SQ FT)	L(FT)	W(FT)	AREA(SQ FT)	L(FT)	W(FT)
300.0	0.975000E+05	570.	171.	0.975000E+05	570.	171.
600.0	0.600000E+05	391.	154.	0.112500E+06	570.	197.
900.0	0.450000E+05	391.	115.	0.150000E+06	695.	216.
1200.0	0.450000E+05	391.	115.	0.187500E+06	828.	227.
1500.0	0.375000E+05	292.	129.	0.225000E+06	966.	233.
800.0	0.225000E+05	212.	106.	0.247500E+06	1107.	224.
2100.0	0.225000E+05	212.	106.	0.270000E+06	1250.	216.
2400.0	0.750000E+04	158.	47.	0.277500E+06	1395.	199.
2700.0	0.000000E+00	0.	0.	0.277500E+06	1395.	199.
3000.0	0.000000E+00	0.	0.	0.277500E+06	1395.	199.
3300.0	0.000000E+00	0.	0.	0.277500E+06	1395.	199.
3600.0	0.000000E+00	0.	0.	0.277500E+06	1395.	199.

Figure C-7. Selected Output for Barge Disposal (concluded).

the bottom before 900 s so there is no history for those sediment fractions. Concentrations for clay are shown to decrease with time for both requested depths and the quarterly time entered in the input data. The lead concentrations are shown for every time step for the two depths of 15 and 39 ft as well as depths 0, 10, 20, 30 and 40 ft. The model evaluates concentrations at five additional depths based on the location of clouds to better estimate the peak concentration of contaminant in the water column. The last section of Figure C-7 shows the snapshot mixing zone (at a instant in time) and accumulated mixing zone (from the beginning of simulation). The snapshot columns show the area that exceeds the water quality standard at the time of the results. For example, at 300s a region 570 ft long, 171 ft wide and 97,500 ft^2 in area has a lead concentration that exceeds the water quality standard. Areas were in violation up to and including 2400 seconds. The accumulated columns show the area that exceeds or previously exceeded the standard at the time of the results. The appropriate required mixing zone has an area of 277,500 ft^2, a length of 1395 ft, and a width of 199 ft.

C2.8.1.2.3 Plots of Concentration Following Barge Disposal

From the "STFATE Activity Selection Menu," "F4-Generate Graphics" is selected to receive the "STFATE Graphics File Selection Menu" which has several options. First, the name of the data file used during execution is entered after selecting "F1-Enter name of data file used during execution". Next, the "F4-Generate graphics with selected file" is pressed to receive the "STFATE Graphics Generation Menu". The first option is to select "F1-Maximum concentrations versus time" which brings up a screen with selections for how the graph is to be displayed (screen, printer, plotter), which solids fraction or contaminant (clumps, sand, clay or lead in this example) is desired, and what depth (15, 39 or peak) are desired. Peak means the water column depths at which the maximum concentrations occur. The maximum concentration versus time for lead in this barge disposal example (Fig. C-8) shows the maximum (0.039 mg/L) occurring 5 min after disposal and rapidly dropping to below the mixing zone standard (0.0032 mg/L) at 41 min. It then stays under the standard for the remainder of the simulation. Referring back to the middle sections of Figure C-7, it can be determined that the depth where the peak occurred is 20 ft.

Selecting the option "F2-Concentration contours in horizontal plane" displays a screen which provides the ability to graphically display the concentration contours of the contaminant or the solids fraction. It also provides capability for graphically displaying the predicted mixing zone required. As before, the graphs can be output to the screen, plotter or printer. Concentration contours are obtained by selecting the solid fraction or contaminant (clumps, sand, clay or lead) and then selecting the depth desired (15, 39, or peak in this example). The user may select default contours (YES) or specify the desired contours (NO). Selecting NO to specify contours and then pressing PAGE DOWN, the desired contours are entered sequentially on the next screen. The water quality standards will already

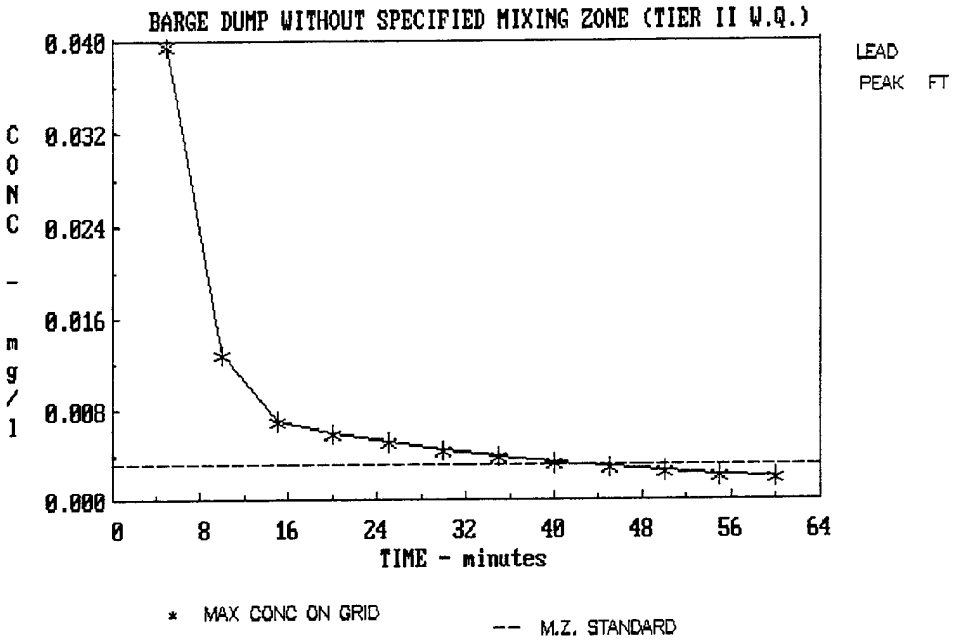


Figure C-8. Peak Lead Concentration in Water Column as a F(Time) for the Barge Disposal Example.

be specified when the mixing zone, contaminant or fluid is selected for display. Figure C-9 shows contours of concentrations of lead above background at a depth of 15 ft after 3600 s. The contour values are specified for contours 1, 2 and 3 as 0.0032, 0.0001 and 0.00005 mg/L. As shown, contour 1 is not displayed in Figure C-9 indicating the concentration value of 0.0032 mg/L above background which is the water quality standard entered in the input file is not exceeded on the grid. For this example, the input requested that the mixing zone be predicted. Figure C-10 shows the predicted "peak" mixing zone outside of which the 0.0032 mg/L standard is not exceeded during the simulation at any depth. To plot the predicted mixing zone, press the F2 key from the "STFATE Graphics Generation Menu" which displays a new screen. Now, select the box "Mixing" and the desired depth. Press PAGE DOWN to receive the next screen and select time and whether defaultcontours are used. In this example, choose NO and press PAGE DOWN. The following screen requests user-specified contour values. Insert contour values and press PAGE DOWN to receive graph. This completes the example and the ESC key is pressed repeatedly to return to the "STFATE Activity Selection Menu" or to quit the program and return to DOS prompt.

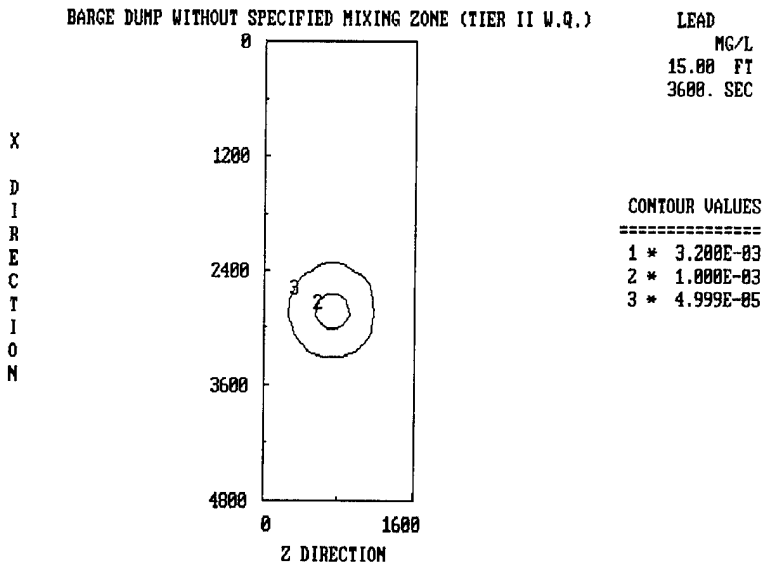


Figure C-9. Lead Concentration Contours for 15 ft Depth at 3600 sec for the Barge Disposal Example.

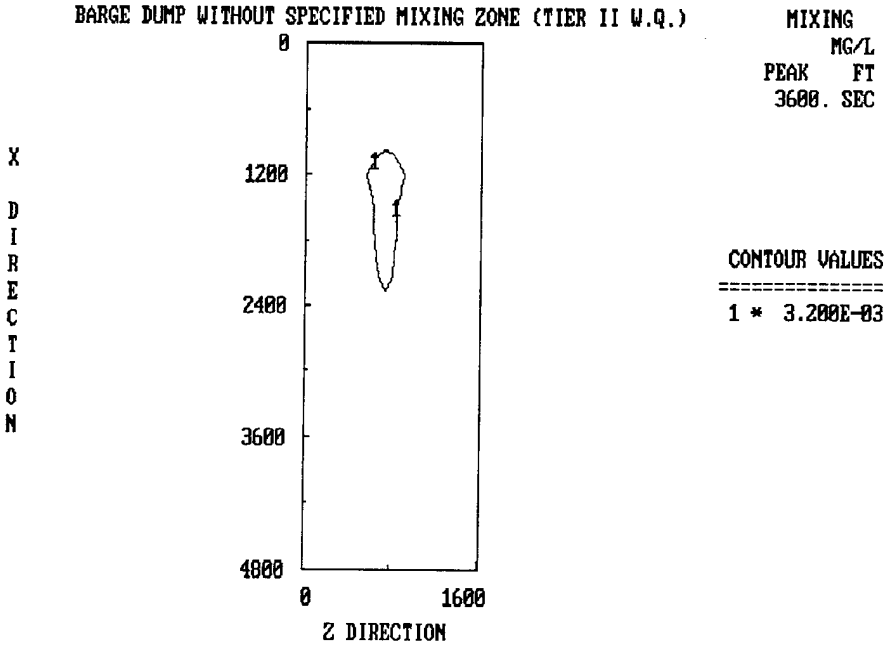


Figure C-10. Plot of Required Mixing Zone for the Barge Disposal Example.

C2.8.2 Multiple-bin Hopper Dredge Example

An example of dredged material disposal is modeled using STFATE for a 3000 yd^3 disposal from a hopper dredge at a constant 40 ft depth site for Section 404(b)(1) regulatory analysis for water column toxicity. A mixing zone is specified for this example, therefore the calculated dredged material concentrations at the boundary of the mixing zone are compared to the allowable concentrations as determined by bioassay tests. A description follows for entering the required example data and the use of the STFATE module.

C2.8.2.1 Entering STFATE and the Input Data File Selection Menu

Many of the steps and procedures for entering the STFATE model for application to a multiple-bin hopper disposal operation are the same as that previously described for the barge disposal (Section 2.8.1.2). Therefore some repetition is contained in this section and a complete description is given for the purpose of clarity.

The STFATE is executed from the DOS prompt, and the "STFATE Activity Selection Menu" is reached as described previously. To proceed, the "Build or edit input data file" option is selected. This results in the "STFATE - Short-Term Fate of a Disposal in Open Water Evaluation Selection Menu" being presented. For this example, the option F3 for "Section 404(b)(1) Reg. Analysis for Navigable Waters" is selected. As a result, the "STFATE Input Data File Selection Menu" appears and key F1 is pressed to enter the name of the input data file to be built or edited. In this example, HOPPER is typed in the highlighted box and then the ENTER key is pressed. Option F4 is then selected to read the input data file if it exists or to initialize it if a new file. A descriptive title, "Example hopper dredge disposal with specified mixing zone (Tier III)," is entered (Press ENTER). The "Disposal Operation Selection Menu" appears and "Disposal from a Multiple-Bin Hopper Dredge" is selected (F2 key) which brings up the "STFATE Input Selection Menu." Entering of the input data given in Table C-4 now begins.

C2.8.2.1.1 Site Description Data

From the "STFATE Input Selection Menu", "F1-Site Description" is selected by pressing key F1 or by using arrow keys to highlight selection and pressing ENTER. On the first data entry screen, the number of grid points is selected as 32 in both the x-direction (top to bottom) and z-direction (left to right). The spacing is picked as 50 ft in the z-direction and 150 ft in the x-direction. These spacings are selected because the water velocity, described later, is 0.5 ft/s in the x-direction and 0.0 ft/s in the

Table C-4. STFATE Input Variables for Section 404(b)(1) Regulatory Analysis of Navigable Waters Using a Multiple-bin Hopper Dredge Disposal.

INPUT PARAMETER	UNITS	INPUT VALUE
SITE DESCRIPTION		
Number of grid points (L-R, +z dir)		32
Number of grid points (T-B, +x dir)		32
Grid spacing (L-R), f(V)	ft	50
Grid spacing (T-B), f(V)	ft	200
Constant water depth	ft	40
Bottom roughness	ft	0.005
Bottom slope (x-dir)	deg	0
Bottom slope (z-dir)	deg	0
Number of points for density profile		2
Density at point one (surface)	g/cc	1.0000
Density at point two (bottom)	g/cc	1.0002
VELOCITY		
Type of velocity profile		1 pt. depth ave., logarithmic
Water depth of averaged velocity	ft	40
Vertically averaged x-dir velocity	ft/s	0.5
Vertically averaged z-dir velocity	ft/s	0
INPUT, EXECUTION & OUTPUT KEYS		
Process to simulate		Disp. from Multiple-bin Hopper Dredge
Duration of simulation	s	3600
Time step for diffusion, f(V)	s	300
Convective descent output		No
Collapse phase output option		No
Number of print times for diffusion		Quarterly
Upper left corner mixing zone	ft	1000, 450
Lower right corner mixing zone	ft	3000, 1050
Toxicity standard for dilution	%	0.4
Number of depths for output		2
Depths of transport-diffusion output	ft	20 / 39
MATERIAL DESCRIPTION		
Number of solids fraction		3
Solid fraction descriptions		sand, silt, clay
Solid fraction specific gravity		2.7, 2.65, 2.65
Solid fraction volume concentration	yd^3/yd^3	0.1, 0.07, 0.04
Solid fraction fall velocity	ft/s	0.1, 0.01, 0.002

(Continued)

Table C-4 (continued). STFATE Input Variables for Section 404(b)(1) Regulatory Analysis of Navigable Waters Using a Multiple-bin Hopper Dredge Disposal.

Solid fraction depositional void ratio		0.6, 3.0, 5.0
Solid fraction critical shear stress	lb/ft^2	0.025, 0.01, 0.002
Cohesive (Y/N)		N, N, Y
Stripped during descent(Y/N)		N, Y, Y
redge site water density	g/cc	1.0
INPUT PARAMETER	**UNITS**	**INPUT VALUE**
DISPOSAL OPERATION		
Disposal point top of grid	ft	1300
Disposal point left edge of grid	ft	750
Length of vessel bin	ft	60
Width of vessel bin	ft	20
Distance between bins	ft	5
Time required to empty all hopper bins	s	60
Number of bins opening simultaneously		2
Number of discrete openings of bins		3
Vessel velocity in x-dir for each set	ft/s	6 / 6 / 6
Vessel velocity in z-dir for each set	ft/s	0 / 0 / 0
Bottom depression length x-direction	ft	0
Bottom depression length z-direction	ft	0
Bottom depression average depth	ft	0
Predisposal draft	ft	18
Postdisposal draft	ft	5
COEFFICIENTS		
Settling coef (BETA)		0.0
Apparent mass coefficient (CM)		1.0
Drag coefficient (CD)		0.5
Form drag collapse cloud (CDRAG)		1.0
Skin friction collapse cloud (CFRIC)		0.01
Drag ellipse wedge (CD3)		0.1
Drag plate (CD4)		1.0
Friction between cloud and bottom (FRICTN)		0.01
4/3 Law horizontal diffusion coefficient (ALAMDA)		0.001
Unstratified vertical diffusion coefficient (AKY0)		0.025
Cloud/ambient density gradient ratio (GAMA)		0.25
Turbulent thermal entrainment (ALPHA0)		0.235
Entrainment collapse (ALPHAC)		0.1
Stripping factor (CSTRIP)		0.003

(Concluded)

z-direction. Thus, the disposal site (Fig. C-11) is 1550 ft wide and 4650 ft long. A constant water depth is entered as 40 ft, and the bottom roughness is input as the mid range value of 0.005. A flat bottom is assumed so a slope of zero is entered for the x- and z-directions. Once the data entry screen is complete, the PAGE DOWN key is pressed to move to the next screen.

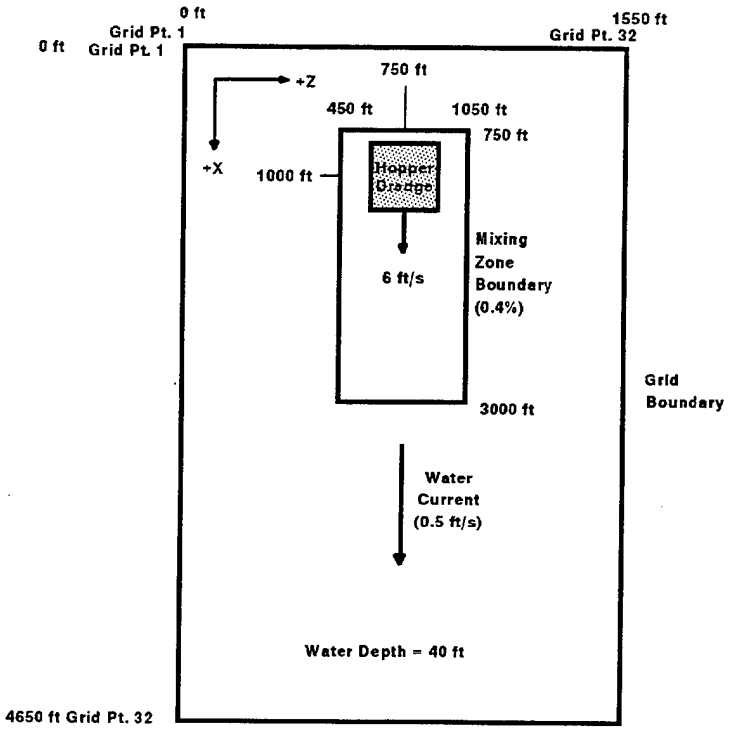


Figure C-11. Schematic of Example Disposal Site for Multiple-bin Hopper Dredge Disposal.

C2.8.2.1.2 Velocity Data for Hopper Disposal Example

The selection of "F2-Velocity Data" from the "STFATE Input Selection Menu" brings up the "Velocity Profile Selection Menu." In this example a depth averaged water velocity profile (Fig. C-12) for a constant depth is selected by pressing the F2 key or highlighting the selection using arrow keys and then pressing PAGE DOWN. The next data entry screen appears on the monitor, and the velocity and constant water depth data are entered in the highlighted boxes. In this case, x-direction velocity of 0.5 ft/s at a depth of 40 ft and z-direction velocity of 0 ft/s are entered. Although zero velocity can be input, it is recommended that the speed of the resultant velocity vector be at least 0.1 ft/s because most open bodies of water have some motion occurring at all times. When the input of velocity data is complete, press PAGE DOWN to return to the "STFATE Input Selection Menu."

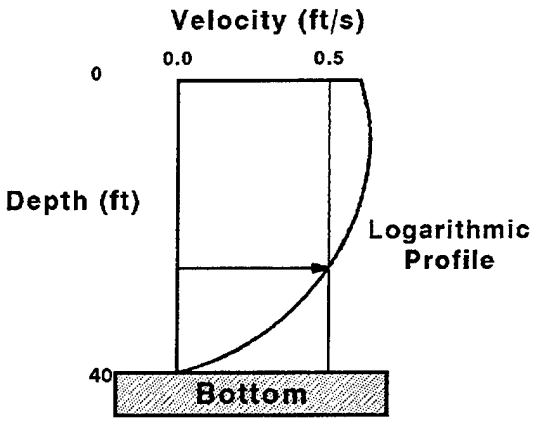


Figure C-12. X-direction Velocity Profile for Hopper Dredge Example.

C2.8.2.1.3 Input, Execution and Output Keys

"F3-Input, Execution and Output Keys" is selected as previously described and the "Simulation Selection Menu" appears. Initial mixing calculations are desired for the hopper disposal so "F3-DESCENT, COLLAPSE AND LONG-TERM DIFFUSION" is selected. Next, the "Evaluation Selection Menu" appears on the monitor, and since this example requires comparison to toxicity results, the "F3-TIER III, COMPARE TOXICITY RESULTS" is chosen. The first input, execution and output keys data entry screen appears and provides for the input of the mixing zone characteristics and the toxicity standard for dilution as a percentage of the initial water column concentration prior to disposal. For this example, the mixing zone upper left corner (x = 750 ft, z = 450 ft) and lower right corner (x = 3000 ft, z = 1050 ft) are entered and the dilution requirement to meet the toxicity standard is appropriately entered. The percent of initial concentration is 0.4%. After entering the data, press PAGE DOWN to receive the next screen. It asks if a zone of initial dilution is desired, and the answer is NO for this example. Press PAGE DOWN to receive the next screen which requests the number of depths (2 to 5) and depths where output on concentrations are desired. In this example 2 depths, 20 and 39 ft, are entered. Using PAGE DOWN, the next data entry screen requests further input concerning the duration of simulation, long-term time step and specifications for printed output. Since the example is for water column evaluation, the one hour (3600 s) duration and a time step of 300 s are input. Output concerning convective descent and collapse phase are not of particular interest for this example, so NO is selected. Also, particular

times for printing time diffusion results are not of importance since summary concentration data are provided for all time steps, so NO is chosen which causes output to be produced quarterly (900, 1800, 2700 and 3600 s). Again, PAGE DOWN is pressed and the "STFATE Input Selection Menu" is returned.

C2.8.2.1.4 Material Description Data

The material description data are entered after choosing the "F4-Material Description Data" from the "STFATE Input Selection Menu." The next data entry screen appears and requests information on the total volume of dredged material in the hopper dredge (total of all bins) and the number of solids fractions in the material. For this hopper disposal example, the total volume is 3000 yd^3, and the number of solid fractions is 3. The next screen is used to enter the physical characteristics of the solids fractions which are entered in the highlighted boxes on the screen. Typical values and their ranges are shown at the top of the screen, and the input values are shown at the bottom of the data entry screen and are tabulated in Table C-4. Press PAGE DOWN to get the next screen which asks if the adjustment of the entrainment and drag coefficients based on the moisture content are desired. Typically, this is not necessary and NO is selected as is done in this example. The final screen for material description requests input on the density of the dredging site water which is in the hopper with the dredged material solids. For the example, the density is entered directly (YES to first question) and the value of 1.000 g/cc is accepted. If the density is different, it can be entered in the highlighted box. If salinity and temperature data are only available, then NO is selected and another screen will appear to allow for the input of that data. At this point, PAGE DOWN is selected and the "STFATE Input Selection Menu" returns.

C2.8.2.1.5 Operation Data

To describe the disposal operation, "F5-Disposal Operation Data" is selected and the first data entry screen is used to enter data concerning location of disposal point, length and width of disposal vessel (hopper) bin, distance between bins, pre- and post-disposal hopper draft, total time needed to empty all bins and the number of bins that are opened simultaneously. A schematic of the hopper dredge bin layout is illustrated in Figure C-13. The actual data are entered into the respective highlighted boxes, and the input data values are given in Table C-4. For this example, the disposal point is 1000 ft from the top edge of grid and 750 ft from left edge of grid (Fig. C-11). The length and width of each is 60 and 20 ft, respectively, and the distance between the edge of bins is 5 ft. The pre- and post-disposal drafts are 18 and 5 ft, respectively, and it takes 60 s to empty all bins. Press PAGE DOWN to get next screen which requests information concerning the number of discrete openings of pairs of hopper bins and the velocity of the hopper dredge. For this example there are three discrete openings of sets of two hopper bins. Also, the hopper dredge is assumed to travel at a constant

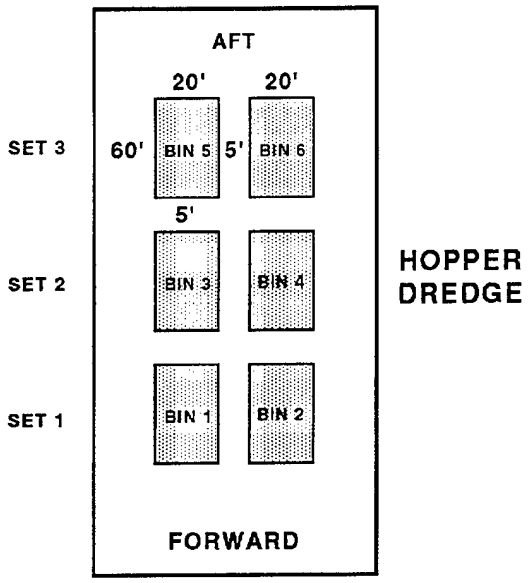


Figure C-13. Schematic of Hopper Dredge with 6 Bins. In this example, the bins are opened in sets of two (bins 1 & 2, bins 3 & 4 and bins 5 & 6).

velocity of 6 ft/s during all three discrete openings. After entering these data and pressing PAGE DOWN, the next screen requires data entry concerning disposal in a pre-existing depression, and for this example values of zero are accepted. PAGE DOWN is pressed again and the "STFATE Input Selection Menu" reappears on the monitor.

C2.8.2.1.6 Coefficients

The "STFATE Input Selection Menu" is now on the monitor and the next selection is "F6-Coefficients (Default Values)". Highlighting this selection and pressing ENTER or pressing F6 displays the numerical model coefficients. In most cases the default values should be chosen and this was done for the example by pressing PAGE DOWN. If other values are required, they may be entered in the appropriate highlighted box before pressing PAGE DOWN. Expert advice should be obtained before using coefficient values different from the default numbers.

C2.8.2.1.7 Saving Input Data Menu

The entering of data is now complete as indicated by the asterisks by each data entry option. The next step is to save the input data file for use in the execution of STFATE. On the "STFATE Input

Selection Menu," press the F7 key or highlight "F7-Saving Input Data Menu" and press ENTER. The "Saving Input Data Menu" appears and requests input as to whether a new file name is desired or the active data file should be used for storing the data. The file name entered at the beginning of the input process appears as the active data file. Sometimes a file is being edited, but the original data file needs to be kept unchanged. At this point a new file name can be selected using option "F1-Enter name of file to be saved" and then option "F4-Save data in (or to) the active data file" is chosen to save the data. For the example, the active data file HOPPER is selected. If the active data file exists, the program indicates so and requests permission to overwrite the file. In this example, "Y" is entered and the next screen requests a descriptive title for the file. The title "Hopper Discharge with specified mixing zone (Tier III)" is entered. Next, the "STFATE Input Selection Menu" reappears and all of the selections show an asterisk indicating each selection has been completed. To complete the process, ESC is pressed and the "STFATE Activity Menu" appears. Now the input file is complete and saved, and the STFATE model can be executed by selecting "F2-Execute STFATE" which then requests the input data file to be entered. HOPPER is entered to obtain results for this example which are discussed int the next section.

C2.8.2.2 Description of Example Hopper Disposal Output

The objective of this section is to illustrate and describe selected portions of the hopper dredge disposal results. Accumulation of sediment on the bottom is not discussed since the emphasis is on results for water column concentrations for Tier II and III evaluations. The results discussed are concentrations of the solids fractions, contaminant and the fluid associated with the dredged material in the hopper bins.

As previously explained in the barge example, results are accessed through the "STFATE Activity Selection Menu". This screen provides two options for output, "F3-Print or view output" and "F4-Generate graphics." If option F3 is selected, the "STFATE Output Data File Selection Menu" appears and provides several possibilities. First, the option "F1-Enter name of data file used during execution" is used, and the filename HOPPER is entered after selecting this option. Once the filename is entered, the options "F4-Print selected output file" or "F5-View selected output file" can be used. There is considerable output from the model and all of the output is usually not desired. The F5 option provides viewing of the output file by paging through it using the PAGE UP and PAGE DOWN keys. The viewing software can be used to select specific portions of the output for printing a hard copy. Pressing the ESC key returns the "STFATE Activity Selection Menu".

C2.8.2.2.1 Hopper Disposal Water Column Concentrations and Area Distribution

In this example, water column concentrations of the solids fractions and the fluid volume ratio (volume of dumped fluid/volume of ambient water) are requested at 20 and 39 ft. Thus, the concentrations for sand, silt, clay and the fluid at every grid point location for both depths are contained in the output file provided the material has not settled to the bottom. In addition, results are also available showing the maximum concentration occurring at each grid point for anywhere in the water column as well as at the requested depths throughout the duration of the simulation. The top portion of the selected output shown in Figure C-14 shows the output for fluid volume ratio (volume of fluid from the discharge / volume of water column at the grid point) at 20 ft at the end of the model run (3600 s). These values at each grid point are multiplied by the appropriate scale factor (0.01) as given at the top of the output. These values would be multiplied by 100 if the results were desired with units of percent. In this example the maximum value on the grid is 1.6 which is multiplied by 0.01 yielding a maximum fluid volume ratio of 0.016 at x- and z-grid locations of 21 and 16 respectively. Recall from the input that the distance between x-grid points is 150 ft and between z-grid points is 50 ft. Therefore the maximum concentration occurs 3000 ft from top of grid and 750 ft from left edge of grid. The disposal began at an x- and z-distance of 1000 ft (grid point 8) and 750 ft (grid point 16). Both the hopper dredge and water velocity were in the positive x-direction so it is reasonable to find the maximum concentration down grid from the disposal point. Since the hopper and the water current are moving in the positive x-direction, it is expected that the distance in the x-direction affected by the discharge should be longer than that in the z-direction. The display of the area with concentrations greater than 10^{-8} vol/vol in Figure C-14 appears to be wider than it is long, but that is because of the difference in grid spacing between the x- and z-direction. It is actually 800 ft wide (z-direction) and 1500 ft long (x-direction).

C2.8.2.2.2 Hopper Water Column Concentrations

The water column concentrations over the duration of the simulation are tabulated in Figure C-14. This shows the clay and percent fluid values versus time. The sand settled to the bottom before 900 s so there is no history for this sediment fraction. Maximum concentrations for silt and clay on the entire grid are shown to decrease with time for both requested depths and the quarterly time entered in the input data while outside the mixing zone the concentrations increased as the plume moved out from the mixing zone. The fluid volume ratio is shown for every time step and at the two depths of 20 and 39 ft as well as the model selected depths of 0, 10, 20, 30, and 40 ft. The simulation

CONCENTRATIONS ABOVE BACKGROUND OF FLUID (VOLUMETRIC RATIO OF DUMP FLUID TO AMBIENT WATER) IN THE CLOUD 3600.00 SECONDS AFTER DUMP

20.00 FT BELOW THE WATER SURFACE

...MULTIPLY DISPLAYED VALUES BY 0.1000E-01 (LEGEND... + = .LT. .01 . = .LT. .0001 0 = .LT. .000001)

M N=	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	3031
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
4	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
5	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
6	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
7	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
8	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
9	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
10	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
11	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
12	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
13	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
14	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
15	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
16	0000	0	0	0	0	0	0	0	0	0	.	.	.	.	.	.	.	.	.	0	0	0	0	0	0	0	0	0	0000
17	0000	0	0	0	0	.	.	.	.	.	+	+	+	+	+	+	+	+	+	.	.	0	0	0	0	0	0	0	0000
18	0000	0	0	0	0	.	+	+	+	+	+	+	.01	.01	.01	.01	.01	+	+	+	+	0	0	0	0	0	0	0	0000
19	0000	0	0	0	0	+	+	+	.01	.03	.06	.09	.13	.16	.17	.16	.13	.09	.06	.03	.01	0	0	0	0	0	0	0	0000
20	0000	0	0	0	0	+	.01	.03	.08	.16	.29	.46	.63	.76	.81	.76	.63	.46	.29	.16	.08	0	0	0	0	0	0	0	0000
21	0000	0	0	0	0	+	.02	.07	.16	.33	.58	.90	1.2	1.5	1.6	1.5	1.2	.90	.58	.33	.16	0	0	0	0	0	0	0	0000
22	0000	0	0	0	0	+	.02	.06	.15	.30	.53	.83	1.1	1.3	1.4	1.3	1.1	.83	.53	.30	.15	0	0	0	0	0	0	0	0000
23	0000	0	0	0	0	+	.01	.02	.06	.13	.23	.35	.48	.59	.62	.59	.48	.35	.23	.13	.06	0	0	0	0	0	0	0	0000
24	0000	0	0	0	0	+	+	+	.01	.02	.04	.06	.08	.10	.10	.10	.08	.06	.04	.02	.01	0	0	0	0	0	0	0	0000
25	0000	0	0	0	0	.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0	0	0000
26	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
27	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
28	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
29	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
30	0000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0000
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

INITIAL MIXING COMPUTATIONS RESULTS FOR SILT:

TIME (HR)	DEPTH (FT)	MAX CONC ABOVE BACKGROUND ON ENTIRE GRID (MG/L)	X-LOC (FT)	Z-LOC (FT)	MAX CONC ABOVE BACKGROUND OUTSIDE MIXING ZONE (MG/L)
0.25	20.0	0.493E+03	1800.	750.	0.504E-04
0.50	20.0	0.456E+03	2100.	750.	0.146E-01
0.75	20.0	0.311E+03	2550.	750.	0.507E+00
1.00	20.0	0.217E+03	3000.	750.	0.217E+03
0.25	39.0	0.137E+04	1650.	750.	0.162E-04
0.50	39.0	0.751E+02	2100.	750.	0.240E-02
0.75	39.0	0.511E+02	2550.	750.	0.834E-01
1.00	39.0	0.357E+02	3000.	750.	0.357E+02

INITIAL MIXING COMPUTATIONS RESULTS FOR CLAY:

TIME (HR)	DEPTH (FT)	MAX CONC ABOVE BACKGROUND ON ENTIRE GRID (MG/L)	X-LOC (FT)	Z-LOC (FT)	MAX CONC ABOVE BACKGROUND OUTSIDE MIXING ZONE (MG/L)
0.25	20.0	0.477E+03	1800.	750.	0.476E-04
0.50	20.0	0.299E+03	2100.	750.	0.215E-01
0.75	20.0	0.209E+03	2550.	750.	0.516E+00
1.00	20.0	0.147E+03	3000.	750.	0.147E+03
0.25	39.0	0.244E+03	1500.	750.	0.214E-04
0.50	39.0	0.492E+02	2100.	750.	0.353E-02
0.75	39.0	0.343E+02	2550.	750.	0.849E-01
1.00	39.0	0.241E+02	3000.	750.	0.241E+02

Figure C-14. Selected Output for Hopper Dredge Disposal.

INITIAL MIXING COMPUTATIONS RESULTS FOR FLUID:

TIME (HR)	DEPTH (FT)	MAX CONC ABOVE BACKGROUND ON ENTIRE GRID (PERCENT)	X-LOC (FT)	Z-LOC (FT)	MAX CONC ABOVE BACKGROUND OUTSIDE MIXING ZONE (PERCENT)
0.08	20.0	0.203E+01	1350.	750.	0.823E-26
0.17	20.0	0.548E+01	1500.	750.	0.305E-05
0.25	20.0	0.485E+01	1650.	750.	0.559E-04
0.33	20.0	0.427E+01	1800.	750.	0.480E-03
0.42	20.0	0.376E+01	1950.	750.	0.240E-02
0.50	20.0	0.331E+01	2100.	750.	0.794E-02
0.58	20.0	0.292E+01	2250.	750.	0.195E-01
0.67	20.0	0.258E+01	2400.	750.	0.386E-01
0.75	20.0	0.228E+01	2550.	750.	0.649E-01
0.83	20.0	0.202E+01	2700.	750.	0.627E+00
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 0.83 HOURS.					
0.92	20.0	0.180E+01	2850.	750.	0.162E+01
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 0.92 HOURS.					
1.00	20.0	0.160E+01	3000.	750.	0.160E+01
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 1.00 HOURS.					
0.08	39.0	0.245E+01	1500.	750.	0.805E-26
0.17	39.0	0.902E+00	1500.	750.	0.503E-06
0.25	39.0	0.797E+00	1650.	750.	0.920E-05
0.33	39.0	0.702E+00	1800.	750.	0.789E-04
0.42	39.0	0.618E+00	1950.	750.	0.395E-03
0.50	39.0	0.544E+00	2100.	750.	0.131E-02
0.58	39.0	0.480E+00	2250.	750.	0.321E-02
0.67	39.0	0.424E+00	2400.	750.	0.634E-02
0.75	39.0	0.375E+00	2550.	750.	0.107E-01
0.83	39.0	0.332E+00	2700.	750.	0.103E+00
0.92	39.0	0.295E+00	2850.	750.	0.267E+00
1.00	39.0	0.263E+00	3000.	750.	0.263E+00
0.08	0.0	0.135E+01	1350.	750.	0.560E-26
0.17	0.0	0.148E+01	1500.	750.	0.735E-06
0.25	0.0	0.131E+01	1650.	750.	0.151E-04
0.33	0.0	0.116E+01	1800.	750.	0.130E-03
0.42	0.0	0.102E+01	1950.	750.	0.649E-03
0.50	0.0	0.896E+00	2100.	750.	0.215E-02
0.58	0.0	0.789E+00	2250.	750.	0.528E-02
0.67	0.0	0.697E+00	2400.	750.	0.104E-01
0.75	0.0	0.617E+00	2550.	750.	0.176E-01
0.83	0.0	0.547E+00	2700.	750.	0.170E+00
0.92	0.0	0.486E+00	2850.	750.	0.439E+00
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 0.92 HOURS.					
1.00	0.0	0.433E+00	3000.	750.	0.433E+00
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 1.00 HOURS.					
0.08	10.0	0.672E+01	1350.	750.	0.251E-25
0.17	10.0	0.338E+01	1500.	750.	0.180E-05
0.25	10.0	0.299E+01	1650.	750.	0.345E-04
0.33	10.0	0.264E+01	1800.	750.	0.296E-03
0.42	10.0	0.232E+01	1950.	750.	0.148E-02
0.50	10.0	0.204E+01	2100.	750.	0.490E-02
0.58	10.0	0.180E+01	2250.	750.	0.120E-01
0.67	10.0	0.159E+01	2400.	750.	0.238E-01
0.75	10.0	0.141E+01	2550.	750.	0.401E-01
0.83	10.0	0.125E+01	2700.	750.	0.387E+00
0.92	10.0	0.111E+01	2850.	750.	0.100E+01
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 0.92 HOURS.					
1.00	10.0	0.989E+00	3000.	750.	0.989E+00
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 1.00 HOURS.					

Figure C-14. Selected Output for Hopper Dredge Disposal. (continued)

```
0.08      20.0      0.122E+02      1500.      750.      0.414E-25
0.17      20.0      0.548E+01      1500.      750.      0.305E-05
0.25      20.0      0.485E+01      1650.      750.      0.559E-04
0.33      20.0      0.427E+01      1800.      750.      0.480E-03
0.42      20.0      0.376E+01      1950.      750.      0.240E-02
0.50      20.0      0.331E+01      2100.      750.      0.794E-02
0.58      20.0      0.292E+01      2250.      750.      0.195E-01
0.67      20.0      0.258E+01      2400.      750.      0.386E-01
0.75      20.0      0.228E+01      2550.      750.      0.649E-01
0.83      20.0      0.202E+01      2700.      750.      0.627E+00
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 0.83 HOURS.
0.92      20.0      0.180E+01      2850.      750.      0.162E+01
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 0.92 HOURS.
1.00      20.0      0.160E+01      3000.      750.      0.160E+01
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 1.00 HOURS.


0.08      30.0      0.798E+01      1500.      750.      0.251E-25
0.17      30.0      0.333E+01      1500.      750.      0.187E-05
0.25      30.0      0.294E+01      1650.      750.      0.339E-04
0.33      30.0      0.259E+01      1800.      750.      0.291E-03
0.42      30.0      0.228E+01      1950.      750.      0.146E-02
0.50      30.0      0.201E+01      2100.      750.      0.481E-02
0.58      30.0      0.177E+01      2250.      750.      0.118E-01
0.67      30.0      0.156E+01      2400.      750.      0.234E-01
0.75      30.0      0.138E+01      2550.      750.      0.393E-01
0.83      30.0      0.123E+01      2700.      750.      0.380E+00
0.92      30.0      0.109E+01      2850.      750.      0.984E+00
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 0.92 HOURS.
1.00      30.0      0.971E+00      3000.      750.      0.971E+00
THE TOXICITY STANDARD IS VIOLATED OUTSIDE THE MIXING ZONE AT 1.00 HOURS.


0.08      40.0      0.167E+01      1500.      750.      0.560E-26
0.17      40.0      0.742E+00      1500.      750.      0.412E-06
0.25      40.0      0.656E+00      1650.      750.      0.757E-05
0.33      40.0      0.578E+00      1800.      750.      0.649E-04
0.42      40.0      0.509E+00      1950.      750.      0.325E-03
0.50      40.0      0.448E+00      2100.      750.      0.107E-02
0.58      40.0      0.395E+00      2250.      750.      0.264E-02
0.67      40.0      0.348E+00      2400.      750.      0.522E-02
0.75      40.0      0.308E+00      2550.      750.      0.878E-02
0.83      40.0      0.273E+00      2700.      750.      0.848E-01
0.92      40.0      0.243E+00      2850.      750.      0.220E+00
1.00      40.0      0.217E+00      3000.      750.      0.217E+00

RESULT:  THE TOXICITY STANDARD WAS VIOLATED OUTSIDE THE MIXING ZONE DURING THE SIMULATION.
```

Figure C-14. Selected Output for Hopper Dredge Disposal. (concluded)

indicates the 0.4% toxicity standard entered in the input phase is violated at depths of 0, 10, 20, and 30 ft, but it is not violated at 39 and 40 ft within the 3600 seconds. Thus the statement at the bottom of Figure C-14 says the toxicity standard is violated outside the mixing zone.

C2.8.2.2.3 Hopper Maximum Concentration and Contour Graphs

From the "STFATE Activity Selection Menu," "F4-Generate Graphics" is selected to receive the "STFATE Graphics File Selection Menu." First, the name of the data file used during execution is entered after selecting "F1-Enter name of data file used during execution." Next, the "F4-Generate graphics with selected file" is pressed to receive the "STFATE Graphics Generation Menu." The first option is to select "F1-Maximum concentrations versus time" which brings a screen up to select where and what to plot. Either the screen, printer or plotter must be selected and then the material (sand, silt, clay or fluid) and depth (20, 39 or peak). Peak means the depth at which the maximum concentrations or fluid ratio occur. The maximum fluid ratio versus time in this hopper disposal example (Fig. C-15) shows the maximum percent (12.2%) on the grid occurs about 5 min after disposal begins and it drops to just below 1.6% near the end of the simulation. The maximum fluid percent outside the mixing zone, defined in the input, is initially below the toxicity standard (0.4%), but the standard is violated at about 49 min after disposal begins and remains in violation for the remainder of the simulation. Referring back to Figure C-14, it can be seen that the peak depth is 20 ft.

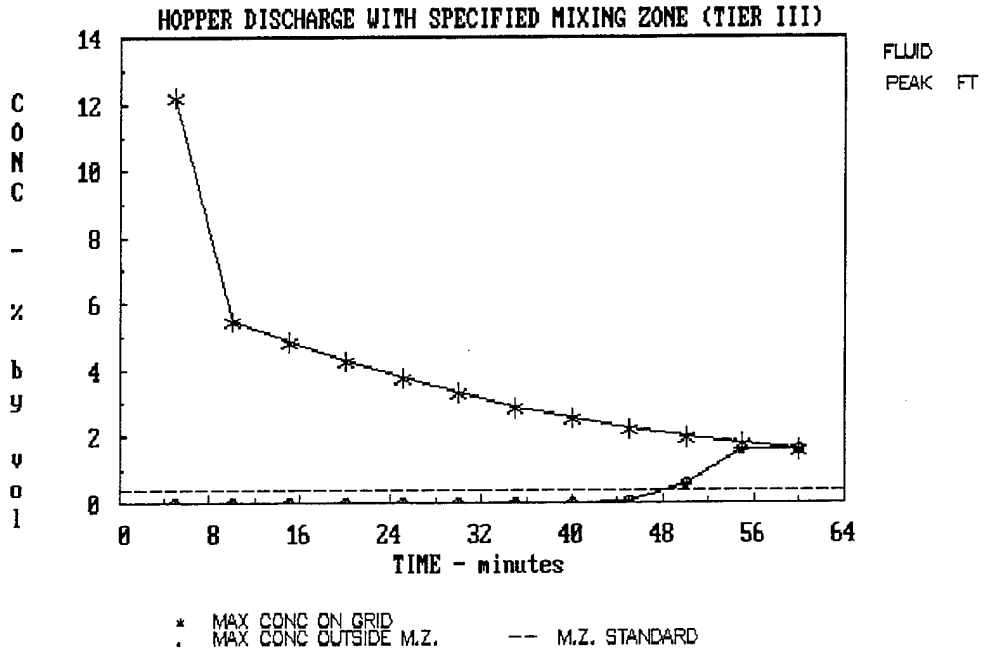


Figure C-15. Peak Fluid Ratio as a F(Time) for the Hopper Dredge Disposal Example.

Selecting option "F2-Concentration contours in horizontal plane" displays a screen which provides the ability to graphically display the percent fluid contours or the solids fraction concentration contours. As before, the graphs can be output to the screen, plotter or printer. The contours are obtained by selecting the solid fraction, fluid or mixing zone and then selecting the depth desired (20, 39, or peak in this example). Default contours are selected by answering "YES" or user-specified contours are selected by answering "NO". Figure C-16 shows one fluid percent contour at 20 ft for 3600 s after initiation of disposal. The contour value was specified for only one contour and the toxicity value (0.4%) was entered. Thus, Figure C-16 shows the standard was exceeded just outside the predefined mixing zone at that time. Figure C-17 shows the required mixing zone boundary up to 3600 s after disposal as outlined by the 0.4% contour. Since this contour falls outside the specified mixing, the figure shows a violation of the standard. The highlighted box with "Mixing" is selected from the screen appearing after pressing the F2 option on the "STFATE Graphics Generation Menu" to obtain this result. The ESC key is now pressed repeatedly to return to the "STFATE Activity Selection Menu."

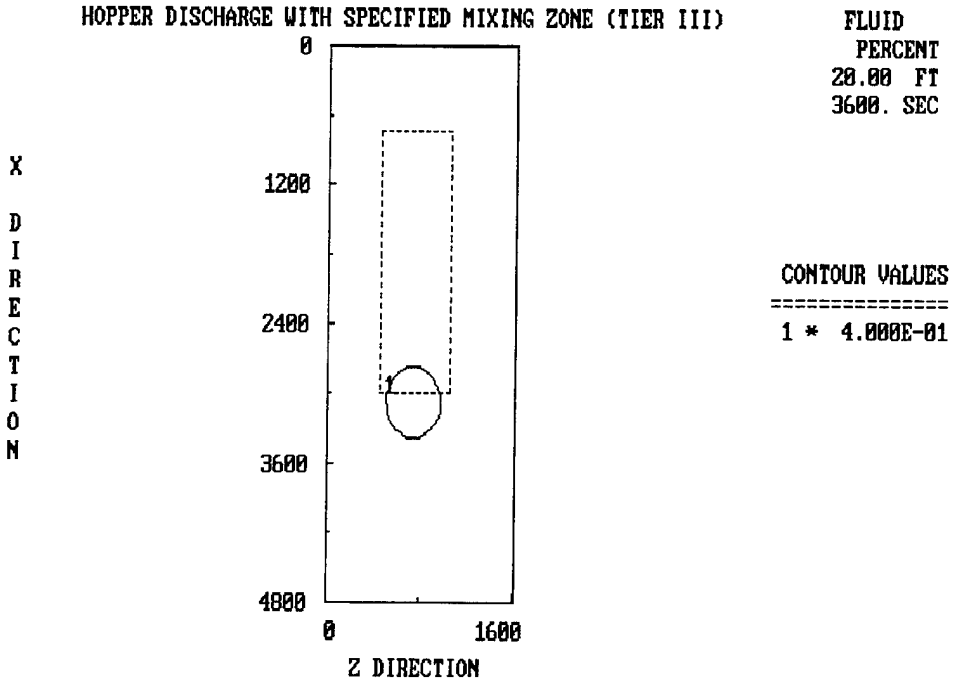


Figure C-16. Fluid Ratio Contours for 20 ft Depth at 3600 sec for the Hopper Dredge Disposal Example.

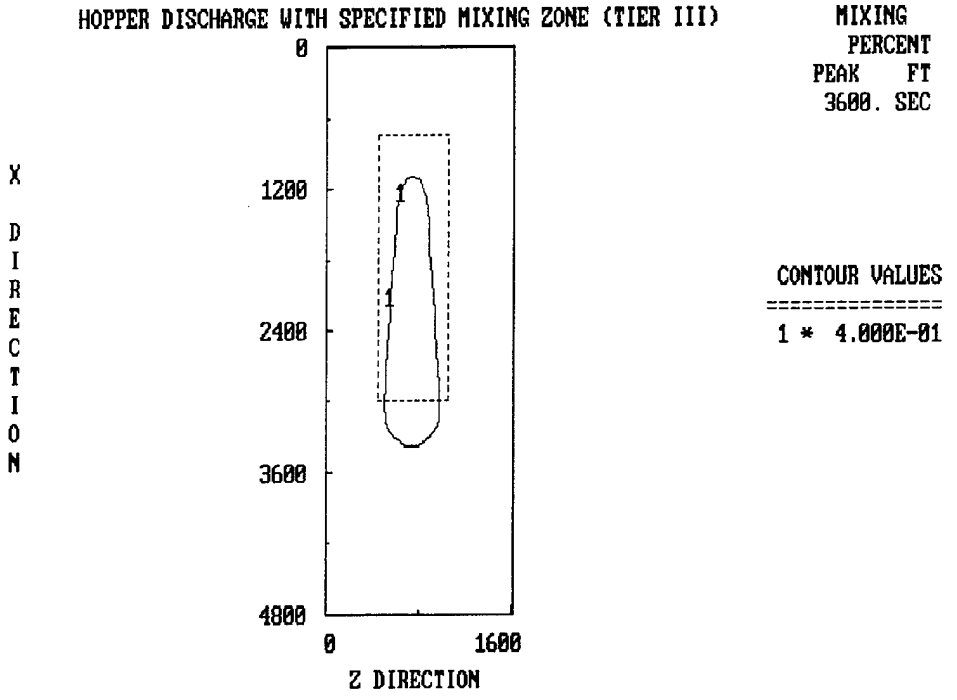


Figure C-17. Plot of Required Mixing Zone for the Hopper Disposal Example.

C3.0 CORNELL MIXING ZONE EXPERT SYSTEM (CORMIX)

The Cornell Mixing Zone Expert System (CORMIX) is a steady state three-dimensional model (Donekar and Jirka, 1990). CORMIX was developed to predict the dilution and trajectory of a submerged single port discharge of arbitrary density (positive, neutral, or negative) into a stratified or uniform-density ambient environment with or without cross-flow. CORMIX is an integral model that accounts for most near-field and some far-field steady state dynamics. CORMIX is presently designed for use in shallow water systems where the jet mixing processes are expected to encounter bottom boundary interaction. CORMIX is capable of representing negatively buoyant plume dynamics through application of mirroring principals; however, the present version does not include sediment settling and deposition.

The current version of the CORMIX model requires some modifications to extend its capabilities to simulate the characteristics of dredged material discharges. Efforts are underway for adaptations of the CORMIX model for simulating the mixing hydrodynamics of several types of dredged material discharges. When these efforts are completed, the revised CORMIX model will be included in subsequent revisions of this appendix[1].

[1] The latest release of CORMIX (Version 2.10) can be obtained without charge from U.S. EPA Office of Research and Development, Center for Exposure Assessment Modeling (CEAM), Athens Environmental Research Laboratory, 960 College Station Road, Athens, Georgia 30605-2720. CORMIX can be either downloaded from CEAM's on-line Bulletin Board System by calling 1-706-546-3402 (FTS 250 3402), or sent through the mail by sending user-supplied diskettes or 9-track magnetic tapes to the CEAM Model Distribution Coordinator at the above address. User documentation is also available from the same source.

C4.0 MACINTYRE ANALYTICAL METHOD FOR CDF DISCHARGE IN RIVERINE CONDITIONS

C4.1 Introduction

This section presents a simplified approach that is applicable to relatively shallow confined riverine water bodies. The method involves a simplistic two-dimensional calculation based on dispersion principles (MacIntyre, 1987). If the mixing-zone size as calculated using simple approximations is within mixing-zone guidelines specified by regulatory agencies, more precise calculations may not be necessary. The mixing-zone calculations depend on a number of assumptions that are difficult to satisfy for estuaries and the tidally influenced portions of rivers. The difficulties are discussed after the presentation of the procedure to be used for a riverine environment.

The analytical solution technique for calculating mixing-zone size described in this section is based on theoretical and empirical relationships for dispersion as summarized by Fischer et al. (1979). Only equations for calculating mixing-zone size resulting from a single-point discharge are presented.

A schematic illustrating a typical single-source effluent discharging into a receiving water body is shown in Figure C-18. For such a condition, the mixing-zone length extends downstream and the body of the mixing zone remains close to the shoreline of the receiving water body.

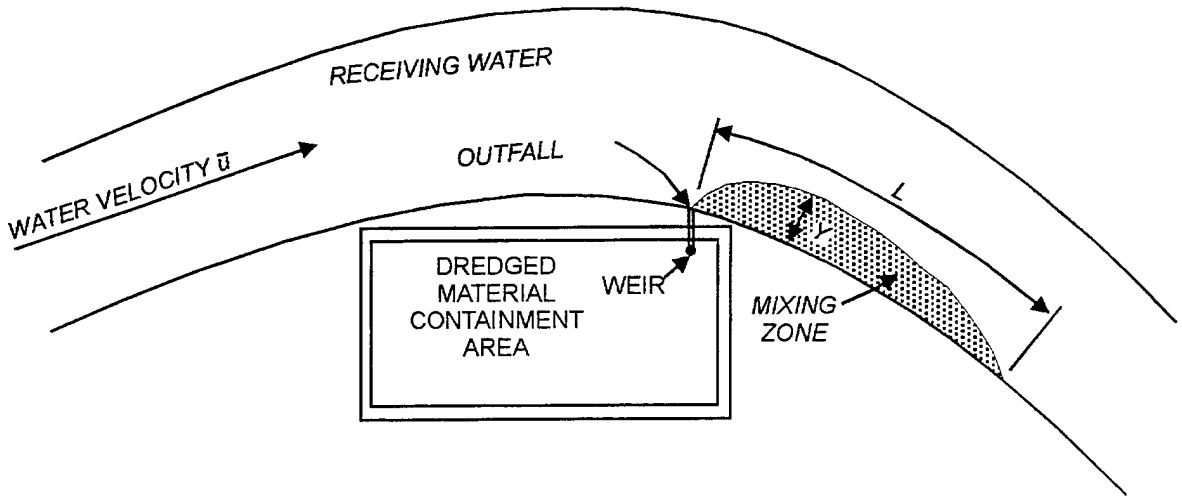


Figure C-18. Schematic of a Mixing Zone for a Single Effluent Source.

C4.2 Data Requirements

The following data are required for evaluating mixing-zone sizes for confined disposal area effluents:

a. Effluent concentrations at the point of discharge and receiving water background concentrations for all contaminants of concern.
b. Water quality standards applicable at the limit of the allowable mixing zone for all contaminants of concern.
c. Depth, cross-sectional area, and current velocity of the receiving water body during expected low flow conditions during the period of dredging.
d. Effluent volumetric flow rate.

C4.3 Calculation Procedure

Step 1. Verify that the assumptions on which the equations depend are reasonable for conditions at the proposed discharge site.

Step 2. Use effluent, receiving water, and water quality standard concentrations of all contaminants of concern to identify the critical contaminant. The critical contaminant is the one that requires the greatest dilution, which will define the boundary of the mixing zone. If mixing evaluations are conducted for toxicity test results the background concentration of dredged material is assumed to be zero and the percentages of dredged material are used to calculate the required dilution.

Step 3. Use receiving-water depth and velocity data to calculate a lateral mixing coefficient. This coefficient is a measure of how rapidly the effluent is dispersed through the receiving water.

Step 4. Calculate mixing-zone length.

Step 5. Check assumptions that depend on mixing-zone length.

Step 6. Calculate the maximum width of the mixing zone.

Step 1 - Assumptions. In order to apply the analytical solution described in this section, the following assumptions are required:

a. No major change in cross-sectional shape, sharp bends, major inflows or outflows, or obstructions to flow exist in the receiving water body in proximity to the mixing zone.
b. The receiving water body can be reasonably approximated by a shallow rectangular cross section.
c. The confined disposal area effluent enters the receiving water as a point source at the bank with negligible horizontal momentum.
d. Differences in density between the effluent and receiving water and in settling rates of suspended particles within the boundary of the mixing zone are negligible.
e. The flow condition in the vicinity of the mixing zone can be approximated as a steady-state velocity flowing parallel to the bank of the receiving water.
f. The major cause of dispersion in the receiving water body is the turbulence and shear flow associated with the horizontal water flow.
g. The effluent plume is vertically well mixed, so that contaminant concentrations do not vary significantly with depth.
h. The width of the effluent plume is small enough that its lateral dispersion is not restricted by the opposite bank of the receiving water body.

Step 2 - Identify critical contaminant. It is necessary to calculate the dilution required within the mixing zone in order to reach applicable water quality standards for all contaminants of concern. This requires an estimate of the effluent concentrations of regulated contaminants. The contaminant that requires the greatest amount of dilution should be calculated as described in Section 5.3.

The maximum boundary of the mixing zone will be defined as the isopleth (line of constant concentration) where the concentration of the most critical contaminant is reduced to the concentration specified by the appropriate water quality standard. It should be noted that if background concentrations exceed the water quality standard, the concept of a mixing zone is inapplicable.

Also, this approach for calculating required dilution is not applicable to turbidity (an optical property of water), which is reduced in an nonlinear fashion by dilution. A correlation curve for TSS versus turbidity should be used to define the TSS concentration corresponding to the water quality standard for turbidity.

Step 3 - Estimate of lateral mixing coefficient.

Step 3.1. The depth of a simplified rectangular cross section for the receiving water body should be calculated as follows:

$$d = \frac{A}{W}$$

where

d = average depth of the receiving water body channel, m

A = cross-sectional area of the channel, m^2

W = surface width of the channel, m

Check to ensure that W is equal to or greater than 10 times the average depth d. If not, the estimate of a lateral mixing coefficient is likely to be inadequate.

Step 3.2. Estimate the shear velocity by one of the following methods. In rivers where the mean channel slope is known, use:

$$u* = \sqrt{gds}$$

In rivers where the channel slope is not known, use:

$$u* = 0.1\ \bar{u}$$

where

u* = shear velocity in receiving water, m/sec^{-1}

g = gravitational acceleration, 9.81 m/sec^{-2}

d = average channel depth, m

S = slope of river bed (dimensionless)

$\bar{u}$ = average of instantaneous velocities across the channel cross section, m/sec^{-1}.

If the flow rate of the receiving water is known, $\bar{u}$ can be calculated as the flow rate divided by the channel cross-sectional area. If the receiving-water flow rate is not known, $\bar{u}$ must be determined from velocity measurements taken at the proposed site. It should be noted that $\bar{u}$ should not be determined over a period of time during which velocity changes occur due to changes in the receiving-water flow rate.

Step 3.3. Estimate the lateral mixing coefficient by using one of the following equations.
In rivers:

$$E_t = 0.3\ du*$$

In estuaries:

$$E_t = 0.4\ du*$$

where

E_t = lateral mixing coefficient, m^2/sec^{-1}

d = average channel depth, m

u* = shear velocity, m/sec^{-1}

The values of lateral mixing coefficient are derived from Fischer et al. (1979) and are based on experimental studies of dispersion in various rivers. Lateral mixing coefficients have been shown to vary widely from one location to another, and the above equations give the lowest reasonable values so that estimates of mixing zone size will be conservative.

Step 4 - Estimate mixing-zone length. If the assumptions presented earlier are valid, the mixing zone will have a shape similar to the one shown in Figure C-18. The length of the mixing zone (measured parallel to the bank) can be estimated as:

$$L = \left(\frac{1}{\pi E_t \overline{u}}\right)\left[\frac{Q_e C_e}{(C_s - C_b)\,d}\right]^2$$

where

L = mixing zone length, m

Q_e = effluent volumetric discharge rate, m^3/sec^{-1}

Step 5 - Check length-dependent assumptions.

Step 5.1. The flow in the water body near the mixing zone can be treated as a steady-state flow as long as:

$$L \leq \frac{\overline{u} T_c}{10}$$

where

L = predicted mixing zone length, m

$\bar{u}$ = cross-sectional average velocity (instantaneous or averaged over a few minutes), m/sec^{-1}

T_c = time taken for the observed value of $\bar{u}$ to change by 10 percent, in seconds

Step 5.2. The lateral dispersion of the effluent plume will not be restricted by opposite bank of the receiving water body as long as:

$$W \geq \sqrt{\frac{8\ E_t L}{\bar{u}}}$$

where W = surface width of receiving water channel, m.

Step 6 - Estimate maximum width of mixing zone. The maximum width of the mixing zone (measured perpendicular to the bank as shown in Figure C-18 can be estimated as:

$$Y = \frac{0.4840 Q_e C_e}{\bar{u}(C_s - C_b)\,d}$$

where Y = maximum width of the mixing zone, m.

C4.4 Example Mixing-Zone Calculation

Following is a hypothetical mixing-zone calculation designed to illustrate the use of the mixing-zone estimation equations. A proposed dredged material containment area is expected to discharge into a river 480 ft (146.3 m) wide. From a study of US Geological Survey stream gage records, it is anticipated that while effluent will be discharged, the lowest river flow will be about 7,600 ft^3/sec (212.8 m^3/sec) and that the river has a cross-sectional area of 4,000 ft^2 (371.6 m^2) at this flow rate. The local bed slope of the river is known to be very variable due to sediment transport. The containment area is expected to have a peak discharge of 15 cfs. The only effluent contaminant that exceeds water quality standards will be cadmium, which is expected to have an effluent concentration of 3.5 µg/L. The background concentration of cadmium in the river is below the detection limit of 0.1 µg/L, and the applicable cadmium water quality standard is 0.25 µg/L. It has been specified that the maximum acceptable mixing-zone size is a 750-ft (228.6-m) radius centered on the effluent outfall.

Step 1 - Assumptions. Since the purpose of this hypothetical problem is to demonstrate the use of the mixing-zone calculations, it has been defined so that all the assumptions on which the calculations depend are valid. Decisions on whether the assumptions are valid depend largely on the professional judgement of personnel familiar with the disposal site.

Step 2 - Identify critical contaminant. Cadmium is the only effluent contaminant that exceeds water quality standards for this example. It is therefore unnecessary to determine the critical contaminant.

Step 3 - Estimate lateral mixing coefficient.

Step 3.1. From the problem statements,

$$A = 4{,}000\ ft^2\ (371.6\ m^2)$$

$$W = 480\ ft\ (146.3\ m)$$

Calculate depth from equation 2:

$$d = \frac{A}{W}$$

$$d = \frac{371.6\ m^2}{146.3\ m} = 2.54\ m$$

Check that $W \geq 10\ d$. It is.

Step 3.2. Since the local bed slope is known to vary due to sediment transport, the shear velocity should be estimated from the mean velocity. Calculate the mean velocity by dividing the river flow of 7,600 ft^3/sec (212.8 m^3/sec) by the cross-sectional area of 4,000 ft^2 (371.6 m^2):

$$\overline{u} = \frac{7{,}600\ cfs}{4{,}000\ ft^2} = 1.90\ ft/sec^{-1}\ (0.579\ m/sec^{-1})$$

and calculate the shear velocity of the receiving waters as follows:

$$u* = 0.1\ \overline{u}$$

$$u* = 0.1(0.579\ m/sec^{-1}) = 0.0579\ m/sec^{-1}$$

Step 3.3. In rivers, the lateral mixing coefficient should be estimated as:

$$E_t = 0.3\ d\ u*$$

$$E_t = 0.3(2.54\ m)(0.0579\ m/sec^{-1})$$

$$E_t = 0.0441\ m^2/sec^{-1}$$

Step 4 - Estimate mixing-zone length. Estimate using the problem statements:

$$Q_e = 15\ cfs\ (0.425\ m^3/sec^{-1})$$

$$C_e = 3.5\ \mu g/L^{-1}\ (3.5 \times 10^{-3} mg/L^{-1})$$

$$C_s = 0.25\ \mu g/L^{-1}\ (2.5 \times 10^{-4}\ mg/L^{-1})$$

$$C_b < 0.1\ \mu g/L^{-1}\ (1.0 \times 10^{-4}\ mg/L^{-1})$$

In order to be conservative, it would be wise to assume that the background concentration is only just under the detection limit, rather than zero. Therefore use:

$$C_b = 1.0 \times 10^{-4}\ mg/L^{-1}$$

Calculate mixing-zone length:

$$L = \left(\frac{1}{\pi E_t \overline{u}}\right)\left[\frac{Q_e C_e}{(C_s - C_b)\,d}\right]^2$$

$$L = \left[\frac{1}{\pi(0.0441\ m^2/sec^{-1})(0.579\ m/sec^{-1})}\right]$$

$$\left\{\frac{(0.425\ m^2/sec)(3.5 \times 10^{-3} mg/L^{-1})}{[(2.5 - 1.0) \times 10^{-4} mg\ L^{-1}](2.54\ m)}\right\}$$

$$L = 190\ m\ (623\ ft)$$

Step 5 - Check length-dependent assumptions.

Step 5.1:

Verify that the flow of the water body near the mixing zone can be treated as a steady state flow.

$$L \leq \frac{\overline{u} T_c}{10}$$

therefore:

$$T_c \geq \frac{10L}{\overline{u}}$$

$$T_c \geq \frac{10(190\ m)}{0.579\ m/sec^{-1}}$$

$$T_c \geq 3{,}280\ sec\ (55\ min)$$

This is acceptable since the river flow will certainly not change by 10 percent in less than 1 hour.

Step 5.2:

$$W \geq \sqrt{\frac{8E_tL}{\bar{u}}}$$

$$W \geq \sqrt{\frac{8(0.0441\ m^2/sec^{-1})(190\ m)}{(0.579\ m/sec^{-1})}}$$

$$W \geq 10.8\ m$$

This condition is amply satisfied since W equals 146 m.

Step 6 - Estimate maximum width of mixing zone. Estimate the maximum mixing zone width as:

$$Y = \frac{0.484\ Q_eC_e}{\bar{u}(C_s - C_b)d}$$

$$Y = \frac{0.484\ (0.425\ m^3/sec^{-1})(3.5 \times 10^{-3}\ mg/L^{-1})}{0.579\ m/sec^{-1}[(2.5 - 1.0) \times 10^{-4}\ mg/L^{-1}](2.54\ m)}$$

$$Y = 3.3\ m\ (10.7\ ft)$$

Since the mixing zone is predicted to have a length of 623 ft (190 m) and a maximum width of 10.7 ft (3.3 m), it is within the allowable limits of 750 ft (228.6 m) from the effluent outfall.

C5.0 FASTTABS MODELING SYSTEM FOR EVALUATION OF HYDRODYNAMIC TRANSPORT

Rivers, reservoirs, and estuaries have been modeled for a number of years using the USACE TABS numerical modeling system. TABS is a family of two-dimensional numerical models that can simulate hydrodynamic, sediment, and constituent transport processes in these water bodies. TABS has been used to simulate far-field dispersion of instantaneous and continuous dredged material discharges. Some independent near-field analysis is usually required. TABS can handle complex geometries and unsteady flow conditions. Either particulate or dissolved phases of dredged material can be modeled.

The TABS system consists of many separate programs that individually address different aspects of the modeling process (Thomas and McAnally, 1990). These include mesh development, geometry input file generation, boundary condition definition, hydrodynamic input file generation, job status monitoring, and post-processing of the results.

A new graphical implementation of TABS (FastTABS) (Lin et al., 1991) has been developed that successfully addresses the need for efficient model setup, execution, and analysis. It is mouse driven with pull down menus and requires a minimum of manual data entry to complete an application from start to finish. FastTABS was designed to allow easy application of each of the models in the TABS system which include hydrodynamics, constituent and sediment transport. The FastTABS software runs on Macintosh and DOS-based personal computers as well as most UNIX workstations. A primer, user's manual, and tutorial are available[2].

[2] A limited government license allows USACE office use of the FastTABS software supplied through the USACE Waterways Experiment Station (WES). Other users may obtain the software from Brigham Young University, (801)-378-5713. The point of contact for additional information is: Mr. David R. Richards, USACE Waterways Experiment Station, ATTN: CEWES-HE-S, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199, (601)-634-2126.

C6.0 DILUTION VOLUME METHOD FOR CDF EFFLUENT DISCHARGES

C6.1 Approach

A simplified approach to evaluation of mixing zones for CDF effluent discharges is presented in this section in which the volume of water required for dilution is expressed as a rate of flow (Environmental Effects Laboratory, 1976). This approach is generally applicable in both riverine and estuarine conditions. However, the approach should only be applied where there is a discrete discharge source such as a conduit or a weir. Since the effluent discharge will occur at a specified rate V_p, the volume of ambient site water per unit time that would be required to dilute the discharge to acceptable levels can be defined as:

$$V_A = V_p D = V_p [(C_e - C_{BG})/(C_{WQ} - C_{BG})]$$

where

V_A = volume of site water/unit time required for dilution, cfs

V_p = rate of effluent discharge, cfs

C_e = concentration of the contaminant in the effluent in µg/L

C_{BG} = background concentration of the contaminant in the disposal site water in µg/L

C_{WQ} = applicable water quality standard for the contaminant in µg/L

It is assumed that the mixing zone associated with an effluent discharge will resemble the shape in Figure C-19. Therefore, once the required volume per unit time has been calculated, the next step is to determine the dimensions of the mixing zone. The required volume per unit time can also be expressed as:

$$V_A = L\ d\ V_w$$

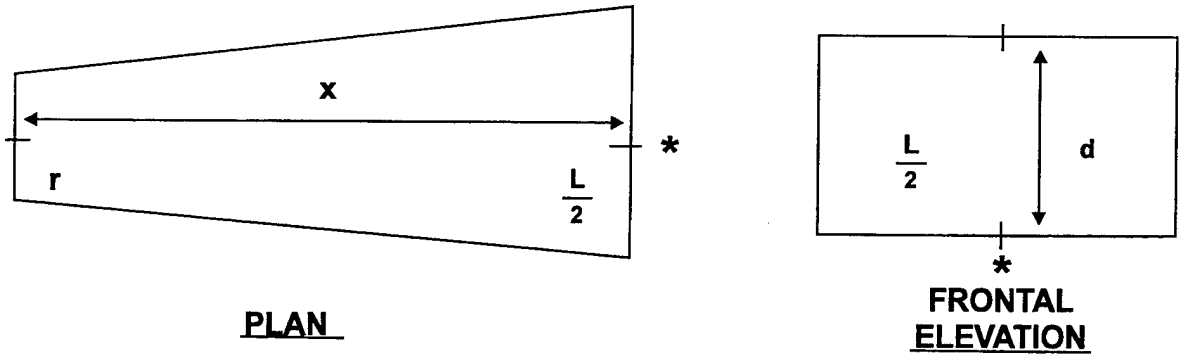
where

V_A = required volume of water per unit time, cfs

L = width of mixing zone at time t, ft

d = depth, ft

V_w = velocity of water at disposal site, ft/sec



PLAN

FRONTAL ELEVATION

PROJECTED SURFACE AREA

$$A = \left(\frac{L}{2} + r\right) X$$

VOLUME PER UNIT TIME

$$V_A = LdV_W$$

Figure C-19. Projected Surface Area and Volume Equations for CDF Effluent Discharge with Prevailing Current.

Since the depth and water velocity are known or can be measured, the width of the front edge of the mixing zone can be calculated as:

$$L = \frac{V_A}{d\ V_w}$$

Based on Brooks (1960) and Johnson and Boyd (1975), the time required for the front edge of the mixing zone to spread laterally to the required width L can be computed from:

$$t = \frac{1}{\lambda} (0.094\ L^{2/3} - 0.149\ r^{2/3})$$

where

t = required time for lateral spreading, sec

L = necessary width of the front edge of mixing zone, ft

r = one-half initial width of the plume at point of discharge (radius of initial surface mixing), ft

λ = turbulent dissipation parameter

Values for λ range from 0.00015 to 0.005 with a value of 0.005 being appropriate in a dynamic environment such as an estuary (Brandsma and Divoky, 1976). As discussed earlier, values for r will be influenced by the method of disposal and will be site specific.

The calculated time can then be used to determine the longitudinal distance the discharge will travel as it is spreading to the required width. This distance can be computed from:

$$X = V_w t$$

where

X = longitudinal movement of discharge, ft

V_w = velocity of water at disposal site, ft/sec

t = necessary time of travel, sec

The results of the previous equations can then be combined to estimate the projected surface area of the proposed discharge. This area can be computed as:

$$A = \frac{L + 2r}{2} X$$

where

A = surface area, ft^2

L = width of front edge of mixing zone, ft

r = radius of initial surface mixing, ft

X = length of the mixing zone, ft

This approach will characterize a proposed discharge by defining the volume of dilution water per unit time that will be required to achieve some acceptable concentration at the edge of the mixing zone. Also, the length and width (and hence the surface area) of the necessary mixing zone will be approximated.

C6.2 Sample Computations

The following computations are presented to illustrate the dilution volume method for a continuous effluent discharge.

The following input values are used in the sample computations:

Volume of effluent discharge per unit time V_p	= 44 cu ft/sec
Turbulent dissipation parameter λ	= 0.005
Water column depth d	= 10 ft
Water velocity V_w	= 0.5 ft/sec
Initial width of plume 2r	= 30 ft
Background concentration C_{BG}	= 0.1 mg/L
Effluent discharge concentration C_e	= 30 mg/L
Applicable water quality standard, C_{wQ}	= 0.5 mg/L

The required volume per unit time will be:

$$V_A = V_p D = 44 \left(\frac{30 - 0.5}{0.5 - 0.1} \right) = 3245 \ cu \ ft/sec$$

The required width of the mixing zone will be:

$$L = \frac{V_A}{d \ V_w} = \frac{3245}{(10) \ (0.5)} = 649 \ ft$$

The time required to achieve the lateral spread L will be:

$$t = \frac{1}{0.005}\left[(0.094)(649)^{2/3} - (0.149)(15)^{2/3}\right]$$

$$= 1228 \text{ sec}$$

The length of the mixing zone will be:

$$X = (0.5 \text{ ft/sec})(1228 \text{ sec}) = 614 \text{ ft}$$

Thus the proposed mixing zone would have dimensions of:

$$\text{Surface area} = \left(\frac{30 + 649}{2}\right) 614 = 208{,}453 \text{ sq ft}$$

$$\text{Maximum dimensions} = 614 \text{ ft by } 649 \text{ ft}$$

This information would be used in considering the compatibility of the size of the mixing zone required to dilute the discharge with the available mixing zone.

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APPENDIX D
STATISTICAL METHODS

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APPENDIX D - STATISTICAL METHODS

D1.0 INTRODUCTION

This Appendix presents the appropriate statistical methods for analyzing data from toxicity 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 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 replications (i.e., containers or aquaria) of the treatments
- 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
- Contaminant concentration data reported as less than detection limit.

Problems such as these, which result in non-ideal data, will be examined and illustrated in detail in an Applications Guide to be published by the USACE as a supplement to this Appendix (Clarke and Brandon, in press).

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

- Tests of assumptions (normality and equality of variances)
- Data-scale transformations
- Two-sample *t*-test
- Nonparametric two-sample test

- Power and sample size calculations
- LC_{50} calculations
- Parametric multiple comparisons among treatments
- Nonparametric multiple comparisons among treatments
- Confidence interval calculations
- Comparisons to action levels

Decision trees are included to provide a general overview of each biological test. These trees illustrate which of the above statistical methods are appropriate for analyzing the results of each biological test, and the order in which the statistical procedures should be conducted. The trees include three general levels of decisions in the biological testing evaluation process: (1) decisions made by evaluating the experimental QA/QC and examining dredged material and reference means, (2) decisions concerning which statistical comparison procedure to use based on tests of assumptions, and (3) decisions concerning the significance of statistical comparisons.

The statistical methods (with the exception of LC_{50} procedures) are illustrated in this Appendix with example data analyzed by SAS IBM-compatible PC programs (SAS Institute, Inc., 1988a-c). This manual does not constitute official endorsement or approval of these or any other commercial hardware or software products. Other equally acceptable hardware and software products are commercially available and may be used to perform the necessary analyses. For example, all analyses required for this Appendix can be conducted using SYSTAT (Steinberg, 1988; Wilkinson, 1990; Steinberg and Colla, 1991), with different tests for normality and equality of variances. If it is necessary to write original programs to perform statistical analysis, the appropriateness of the techniques and accuracy of the calculations must be very carefully verified and documented.

Each example data set included in this Appendix is analyzed using several different statistical methods (usually, all of the possible tests in the appropriate decision tree) for illustrative purposes only. *Note that the results of different statistical tests will occasionally disagree, and it is never appropriate to conduct several tests in order to choose the result one likes best.* Decisions concerning the proper statistical tests to use should be made *a priori*, based on such considerations as experimental design, hypotheses of interest, relative importance of Type I and Type II error rates (Section D1.2), and tests of assumptions (Section D2.1.1.1).

D1.1 Basic Statistics

Statistical methods are used to make inferences about *populations*, based on *samples* from those populations. In most toxicity and bioaccumulation tests, samples of exposed organisms are used to estimate the response of the population of laboratory organisms. The response from the samples is usually compared with the response to a reference[1], or with some fixed standard such as an FDA action level. In any toxicity or bioaccumulation test, summary statistics such as means and standard errors for response variables (e.g., survival, contaminant levels in tissue) should be provided for each treatment (e.g., elutriate concentration, sediment).

In the tests described herein, samples or observations refer to *replicates* of treatments. Sample size n is the number of replicates (i.e., experimental units, test containers) in an individual treatment, not the number of organisms in a test container. Overall sample size N is the total number of replicates in all treatments combined, i.e.,

$$N = n_1 + n_2 + n_3 + \ldots + n_k$$

where k is the total number of treatments in the experiment.

The statistical methods discussed in this Appendix are described in general statistics texts such as Steel and Torrie (1980), Sokal and Rohlf (1981), Dixon and Massey (1983), Zar (1984), and Snedecor and Cochran (1989). We recommend that investigators using this Appendix have at least one of these texts on hand. A nonparametric statistics text such as Conover (1980) can also be helpful.

Mean

The sample mean ($\bar{x}$) is the average value, or $\Sigma x_i / n$, where

n = number of observations (replicates)
x_i = ith observation, e.g., x_2 is the second observation
Σx_i = every x summed = $x_1 + x_2 + x_3 + \ldots + x_n$; *usually written* Σx

Most calculators and statistical software packages will provide means.

Standard deviation

The sample standard deviation (s) is a measure of the variation of the data around the mean. The sample variance, s^2, is given by:

[1] Reference is used generically to refer either to a reference sediment (as in benthic toxicity and bioaccumulation testing), or to dilution water or control water (used in water column toxicity testing).

$$s^2 = \frac{\Sigma x^2 - (\Sigma x)^2/n}{n - 1} \quad \text{(Eq. 1)}$$

Standard error of the mean

The standard error of the mean (SE, or $s/\sqrt{n}$) estimates variation among sample means rather than among individual values. The SE is an estimate of the SD among means that would be obtained from several samples of n observations each. Most of the statistical tests in this manual compare means with other means (e.g., dredged sediment mean with reference mean) or with a fixed standard (e.g., FDA action level). Therefore, the "natural" or "random" variation of sample means (estimated by SE), rather than the variation among individual observations (estimated by s), is required for the tests.

In addition to the summary statistics above, two other statistics derived from the normal (bell-shaped) frequency distribution are central to statistical testing and to the tests described in this Appendix. These two statistics are normal deviates (z-scores) and Student's t.

Normal deviates (z)

Z-scores or normal deviates measure distance from the mean in standard deviation units in a normal distribution. For example, a point 1 standard deviation greater than the mean has a z-score of 1; the mean has a z-score of 0. Z-scores are usually associated with a cumulative probability or proportion. For example, suppose an investigator wants to know the proportion of values in a normal distribution less than or equal to the mean plus 1 standard deviation. In this situation z=0.84, i.e., in a normal distribution 84% of values will be less than or equal to the mean plus 1 standard deviation. Alternatively, an investigator may want to determine the z-score associated with a specific proportion or probability. For example, he or she may want to know the range in which 95% of the values in a normal distribution should fall. That range is the mean ± 1.96 standard deviation (z-scores from -1.96 to +1.96).

Tables of z-scores can be found in most statistical texts, and bear titles such as "Standard Normal Cumulative Probabilities," "Ordinates of the Normal Curve," or "Normal Curve Areas." Typically the z-scores are listed in the column (top) and row (left) margins, with the column marginal value being added to the row marginal value to obtain the z-score. The body of the table contains the probability associated with each z-score. However, depending on the table, that probability may refer to the proportion of all values less than the z-score, the proportion of values falling between 0 and the z-score, or the proportion of values greater than the z-score. For example, if the z-score is 1.96, 97.5% of the values in a normal distribution fall below the z-score (Kleinbaum and Kupper, 1978, Table A-1), 47.5% fall between 0 and the z-score (Rohlf and Sokal, 1969, Table P), and 2.5% fall above the z-score (Steel and Torrie, 1980, Table A.4). It is important to distinguish which probability is of interest.

Z-scores can also be obtained from functions in statistical software packages. For example, in SAS the PROBIT function will return a *z*-score for a specified probability, and the PROBNORM function will compute the proportion of values less than a given *z*-score.

Student's *t*

Normal deviates can only be used to make inferences when the standard deviation is known, rather than estimated. The true population mean (μ) and standard deviation (σ) are only known if the entire population is sampled, which is rare. In most cases samples are taken randomly from the population, and the *s* calculated from those samples is only an estimate of σ. Student's *t*-values account for this uncertainty, but are otherwise similar to normal deviates. For example, an investigator may want to determine the range in which 95% of the values in a population should fall, based on a sample of 20 observations from that population. If the sample consisted of the entire population, μ and σ would be known with certainty, and normal deviates would be used to estimate the desired range (as in the above paragraph). However, if the sample represented only a small proportion of the population, *t*-values would be used to estimate the desired range. The degrees of freedom for the test, which is defined as the sample size minus one (n-1), must be used to obtain the correct *t*-value. Student *t*-values decrease with increasing sample size, because larger samples provide a more precise estimate of μ and σ. For a probability of 95%, the appropriate range of *t*-values is -2.09 to +2.09. In other words, 95% of the values in the population should lie within the range: sample mean ±2.09 *s*. Note that this is wider than the corresponding range calculated using normal deviates. As sample size increases, *t*-values converge on the *z*-scores for the same probability.

Tables of *t*-values typically give the degrees of freedom (df or ν) in the row (left) margin and probabilities or percentiles in the column (top) margin. Percentiles refer to the cumulative proportion of values less than *t*, whereas probabilities (also known as α in this case) refer to the proportion of values less than -*t* and/or greater than +*t*. A two-tailed probability refers to both "tails" of the *t*-distribution curve, i.e., the probability of a value either >+*t* or <-*t*. A one-tailed probability refers to only one of the tails of the curve, e.g., the probability of a value >+*t*.

When using a *t* table, it is crucial to determine whether the table is based on one-tailed probabilities (such as Table V in McClave and Dietrich, 1979, and Table A-2 in Kleinbaum and Kupper, 1978), or two-tailed probabilities (such as Table A.3 of Steel and Torrie, 1980). Some tables give both (such as Table B.3 of Zar, 1984). For most applications involving *t*-values in this Appendix, one-tailed probabilities are desired. The body of the table contains the *t*-value for each df and percentile (or α). The *t*-value for a one-tailed probability may be found in a two-tailed table by looking up *t* under the column for twice the desired one-tailed probability. For example, the one-tailed *t*-value for $\alpha = 0.05$ and df = 20 is 1.725, and is found in a two-tailed table using the column for $\alpha = 0.10$.

Statistical software packages may also provide functions to determine *t*-values or their associated probabilities. In SAS, these functions are TINV and PROBT.

D1.2 Hypothesis Testing

The goal in analyzing toxicity and bioaccumulation test data is to determine whether the mean effect of exposure to a dredged sediment is significantly greater than the mean effect of exposure to a reference. Two formal hypotheses underlie the statistical analysis of data in the two-sample situation. Let μ_R denote the mean effect of exposure to the reference R and let μ_D denote the mean effect of exposure to the dredged sediment D. Then, these two hypotheses are defined as follows:

Null hypothesis

Case 0: H_0: $\mu_D = \mu_R$

There is no difference in mean effect between the treatment (dredged sediment) and reference.

Alternative hypotheses

Case 1: H_1: $\mu_D < \mu_R$

The mean effect of the dredged sediment is less than the mean effect of the reference (e.g., survival).

OR

Case 2: H_1: $\mu_D > \mu_R$

The mean effect of the dredged sediment is greater than the mean effect of the reference (e.g., bioaccumulation).

Our hypothesis test will either reject H_0 for H_1 (Case 1 or Case 2), or will be unable to reject H_0 (Case 0). A one-tailed test is used because there is little concern about identifying a lesser negative effect from the dredged sediment than from the reference.

In performing the hypothesis test, and in determining the sample size to use in the test, the investigator must be aware of the probabilities for two types of errors that can occur in the conclusion. Type I errors occur if, after analysis of the data, H_0 is rejected when it was actually true. In Case 1 for example, a Type I error occurs when it is concluded that the mean effect (e.g., survival) of the dredged sediment is less than the mean effect of the reference when, in fact, the true mean effect of the dredged sediment is not less than that for the reference. Type II errors occur when H_0 is not rejected when it actually should have been rejected (e.g., in Case 2, it is concluded that there is no difference in mean effects of the dredged sediment and reference when, in fact, the true mean effect of the dredged sediment is greater than that of the reference).

To be environmentally protective in dredged sediment disposal evaluations, it is more important to guard against Type II errors. A Type II error could result in inappropriate placement of dredged sediment in the aquatic environment, while a Type I error could result in more costly alternatives to aquatic disposal. The probability of a Type I error is often represented by the letter α; the probability of a Type II error is often written as β. The

significance level or confidence level of a statistical test is 1 - α. The power of a test is 1 - β, which is the probability of rejecting H_0 when it should be rejected, or in other words, the power to detect true significant differences. For example, in Case 2 above, the power is the probability of concluding that the mean effect is greater in the dredged-sediment group when, in fact, this is true. The types of errors and their associated probabilities are summarized in Table D-1.

Table D-1. Types of Errors in Hypothesis Testing and Associated Probabilities.

Hypothesis Test Conclusion	**True State of Nature**	
	H_0 True	H_0 False
H_0 True (do not reject)	Correct (probability = 1 - α)	Type II Error (probability = β)
H_0 False (reject)	Type I Error (probability = α)	Correct (probability = 1 - β)

In hypothesis testing, the Type I error rate is usually prespecified (biological tests, by convention, generally set $\alpha = 0.05$, although there is nothing magical about this probability). An ideal statistical procedure for hypothesis testing seeks to maintain the predetermined α, while minimizing the Type II error rate (i.e., maximizing power). It may not be possible to do both, particularly if the sample data depart from a normal distribution. A test that does well in maintaining the predetermined α, regardless of the characteristics of the sample data, is considered "robust." Tests included in this Appendix were chosen primarily on the basis of power rather than robustness, as the consequences of Type II error were considered more severe than those of Type I error.

Simple formulae for calculating the power of the statistical tests used in this Appendix are presented along with the descriptions of the tests in Sections D2.1.1.1, D2.2.1, D2.2.2, D3.1.2, and D3.2.2. The formulae may be used to calculate the sample size required to ensure a specific power of detecting an effect of a given magnitude (effect size), assuming that effect exists. The formulae can also be used to calculate the power of a specific sample size to detect a specified difference. This latter approach is often more relevant than calculating required sample sizes because budget or logistical constraints usually limit the number of replicates that can be used in biological tests. This is especially true if the tests include expensive chemical analyses (e.g., Tiers III and IV bioaccumulation tests).

D1.3 Experimental Design

Once the investigator has formulated the null hypotheses to be tested, decided upon significance (α) and power (1-β) levels for hypothesis testing, and determined the sample size necessary to achieve the desired power, the next step is to design an experiment to test the hypotheses. Instructions for setting up and conducting sediment toxicity and bioaccumulation experiments are outlined in Chapters 11 and 12, but it is important at this point

to review the basic principles of *experimental design*. These principles include replication, randomization, interspersion, and controls (Hurlbert, 1984).

Replication refers to the assignment of a treatment to more than one experimental unit. The number of replicates, as stated earlier, is the sample size for that treatment. Recall that an experimental unit or replicate is the test container (e.g., a beaker or an aquarium), *not* an individual organism in the test container. The number of organisms in the test container is important only in terms of constituting an adequate measure of the endpoint being tested (e.g., providing sufficient tissue to measure contaminant bioaccumulation). Replication of treatments is necessary to control for random error in the conduct of the experiment. Appendix E includes guidelines for minimum number of replicates for various Tier III and IV bioassays. However, we strongly recommend determining sample size *a priori* using the power formulae in Sections D2.1.1.1, D2.2.1, D2.2.2, and D3.2.2. In many cases, the number of replicates necessary for a powerful statistical test will be greater than the minimum guidelines.

Randomization and interspersion refer to the actual placement of experimental units in the laboratory setup. A random numbers table, available in most statistical texts, may be used to randomly assign treatments to the experimental units. If the randomization does not achieve a reasonable interspersion of treatments, e.g. if several experimental units of the same treatment are clumped together, then a new randomization should be tried. Randomization and interspersion are necessary to control for investigator bias, for initial or inherent variability among experimental units, and for variability in environmental conditions such as lighting, water flow, etc.

Replication, randomization, and interspersion all function to control extraneous sources of variability in an experiment. In addition, *control treatment(s)* are needed to control temporal or procedural variability. In the broadest sense, the control treatment is simply the treatment against which the other treatments are compared. This is the dilution water (or control water) in water column toxicity testing, and the reference sediment in benthic toxicity and bioaccumulation testing. Laboratory controls, such as a clean sand exposure in bioaccumulation testing, may also be included. In Tiers III and IV testing, laboratory controls are used for quality assurance, and are not included in the statistical analyses.

Testing in Tiers III and IV can in most cases be best accomplished using simple experimental designs, either a completely randomized design or a randomized complete blocks design. These designs are discussed in most general statistics texts. In a completely randomized design, treatments are assigned to experimental units randomly over the entire experimental setup. A randomized complete blocks design should be used when the experimental units are placed on or in several different tables, benches or water baths (i.e., "blocks"). Each block holds a certain proportion of the experimental units. Treatments are assigned to experimental units randomly within each block, and each block contains an equal number of replicates of each treatment. Either of these designs is acceptable, providing the principles of replication, randomization, interspersion, and controls are followed. Adherence to the principles of experimental design ensures that the most basic assumption of statistical hypothesis testing, the assumption that treatments are sampled independently, is met.

D2.0 BIOLOGICAL EFFECTS

D2.1 Tier III Water Column Toxicity Tests

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 sediment constituents. If reduced survival is evident, then the median lethal concentration (LC_{50}) or effective sublethal concentration (EC_{50}) of the dredged sediment is calculated from the serial dilution experiment described in Section 11.1.4. Figures D-1 and D-2 provide an overview of water column toxicity test data analysis. Control survival must be ≥90% or some other appropriate value, otherwise the test must be repeated (Section 13.3.17.3). 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% elutriate concentration. When the dilutions are prepared with other than control water, the dilution water treatment is preferred over the control water for the data analysis. If the elutriate survival exceeds the control survival, then the toxicity test indicates no adverse impact from the dredged sediment (Section 11.1.5).

D2.1.1 Comparison of 100% Elutriate and Dilution Water

D2.1.1.1 Methods

Two-sample *t*-test

The usual statistical test for comparing two independent samples such as the 100% elutriate and the dilution water is the two-sample *t*-test (Snedecor and Cochran, 1989). The *t*-test will also be used in some circumstances in benthic toxicity and bioaccumulation tests, to compare individual dredged sediments with a reference (see Figures D-1, D-4A, D-5A).

The *t*-statistic for testing the equality of means $\bar{x}_1$ and $\bar{x}_2$ from two independent samples with n_1 and n_2 replicates is:

$$t = (\bar{x}_1 - \bar{x}_2) \,/\, \sqrt{s^2_{pooled}\,(1/n_1 + 1/n_2)}\,, \qquad \textbf{(Eq. 2)}$$

where s^2_{pooled}, the pooled variance, is calculated as:

$$s^2_{pooled} = [s_1^2(n_1 - 1) + s_2^2(n_2 - 1)] \,/\, (n_1 + n_2 - 2)\,, \qquad \textbf{(Eq. 3)}$$

and where s_1^2 and s_2^2 are the sample variances of the two groups. If the sample sizes are equal ($n_1 = n_2$), then:

$$s_{pooled}^2 (1/n_1 + 1/n_2) = 2s_{pooled}^2 / n \quad . \qquad \textbf{(Eq. 4)}$$

The calculated t is compared with the Student t distribution with $n_1 + n_2 - 2$ degrees of freedom.

The use of Eq.2 to calculate t assumes that the variances of the two groups are equal. If the variances are unequal (see Tests for Equality of Variances below), t is computed as:

$$t = (\bar{x}_1 - \bar{x}_2) / \sqrt{s_1^2/n_1 + s_2^2/n_2} \quad . \qquad \textbf{(Eq. 5)}$$

This statistic is compared with the Student t distribution with degrees of freedom given by Satterthwaite's (1946) approximation:

$$df = \frac{(s_1^2/n_1 + s_2^2/n_2)^2}{(s_1^2/n_1)^2 / (n_1 - 1) + (s_2^2/n_2)^2 / (n_2 - 1)} \quad . \qquad \textbf{(Eq. 6)}$$

This formula can result in fractional degrees of freedom, in which case one should round df down to the nearest integer in order to use a t table. The degrees of freedom for the t-test for unequal variances will usually be less than the degrees of freedom for the t-test for equal variances.

Tests of Assumptions

The two-sample t-test for equal variances (and other parametric tests such as analysis of variance) is only appropriate if:

- there are independent, replicate experimental units for each treatment,
- each treatment is sampled from a normally distributed population, and
- variances for both treatments are equal or similar.

The first assumption is an essential component of experimental design (Section D1.3). The second and third assumptions can be tested using the data obtained from the experiment. Therefore, prior to conducting the t-test, tests for normality and equality of variances should be performed. In some statistical software packages, these

tests of assumptions are done in conjunction with *t*-tests or as part of data summary or screening routines that also provide means, *s*, SE and various diagnostic statistics.

Outliers (extreme values) and systematic departures from a normal distribution (e.g., a log-normal distribution) are the most common causes of departures from normality and/or equality of variances. An appropriate transformation will normalize many distributions. In fact, the arcsine transformation (arcsine, in radians, of $\sqrt{p}$, where p is the survival expressed as a proportion) is so effective, and so frequently necessary, that this Appendix recommends applying it automatically to all survival data in the analysis of toxicity tests. Problems with outliers can usually be solved only by using nonparametric tests, but careful laboratory practices can reduce the frequency of outliers.

Tests for Normality

The most commonly used test for normality for small sample sizes (<50 observations total) is the Shapiro-Wilk's Test. This test determines if residuals are normally distributed. Residuals are the differences between individual observations and the treatment mean. Residuals, rather than raw observations, are tested because subtracting the treatment mean removes any differences among treatments. This scales the observations so that the mean of residuals for each treatment and over all treatments is zero. The Shapiro-Wilk's Test provides a test statistic *W*, which is compared to values of *W* expected from a normal distribution. *W* will generally vary between 0.3 and 1.0, with lower values indicating greater departure from normality. Because normality is desired, one looks for a high value of *W* with an associated probability greater than the prespecified α level.

Table D-2 provides α levels to determine whether departures from normality are significant. Normality should be rejected when the probability associated with *W* (or other normality test statistic) is less than α for the appropriate total number of replicates (*N*) and design. A balanced design means that all treatments have an equal (or nearly equal) number of replicate experimental units. For applications in this Appendix, a design may be considered unbalanced when the treatment with the largest number of replicates (n_{max}) has at least twice as many replicates as the treatment with the fewest replicates (n_{min}). Note that higher α levels are used when number of replicates is small, or when the design is unbalanced, because these are the cases in which departures from normality have the greatest effects on *t*-tests and other parametric comparisons. If data fail the test for normality, even after transformation, nonparametric tests should be used (see Nonparametric Tests below).

Table D-2. Suggested α Levels to Use for Tests of Assumptions.

Test	Number of Observations[a]	α When Design Is	
		Balanced	Unbalanced[b]
Normality	N = 3 to 9	0.10	0.25
	N = 10 to 19	0.05	0.10
	N = 20 or more	0.01	0.05
Equality of Variances	n = 2 to 9	0.10	0.25
	n = 10 or more	0.05	0.10

[a] N = total number of observations (replicates) in all treatments combined; n = number of observations (replicates) in an individual treatment

[b] $n_{max} \geq 2n_{min}$

Tables of quantiles of W can be found in Shapiro and Wilk (1965), Gill (1978), Conover (1980), USEPA (1989) and other statistical texts. These references also provide methods of calculating W, although the calculations can be tedious. For that reason, computer programs are preferred for the calculation of W. SAS can calculate W using the NORMAL option in PROC UNIVARIATE (see Program WATTOX.SAS in Section D4.1).

The Kolmogorov-Smirnov (K-S) Test is also an acceptable test for normality for small sample sizes, provided that the probabilities developed by Lilliefors (1967) are used (Sokal and Rohlf, 1981). The SYSTAT NPAR module provides the appropriate test, and specifically identifies the test as Lilliefors Test (Wilkinson, 1990). Other statistical packages providing K-S Tests may not use the Lilliefors probabilities, and the package documentation should always be checked to determine if the appropriate probabilities are provided. The chi-square (χ^2) test for normality can be used for larger sample sizes (e.g., $N > 50$) (Sokal and Rohlf, 1981).

Tests for Equality of Variances

There are a number of tests for equality of variances. Some of these tests are sensitive to departures from normality, which is why a test for normality should be performed first. Bartlett's Test, Levene's Test, and Cochran's Test (Winer, 1971; Snedecor and Cochran, 1989) all have similar power for small, equal sample sizes (n=5) (Conover et al., 1981), and any one of these tests is adequate for the analyses in this Appendix. Many software packages for t-tests and analysis of variance (ANOVA) provide at least one of the tests. Levene's Test can easily be performed by comparing the absolute values of residuals between treatments using t-tests or ANOVA. SAS statements for conducting Levene's Test are provided in BENTOX.SAS, BIOACC.SAS and BIOACCSS.SAS programs (Sections D4.2.1, D4.3.1 and D4.4.1).

If no tests for equality of variances are included in the available statistical software, Hartley's F_{max} can easily be calculated:

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

When F_{max} is large, the hypothesis of equal variances is more likely to be rejected. F_{max} is a two-tailed test because it does not matter which variance is expected to be larger. Some statistical texts provide critical values of F_{max} (Winer, 1971; Gill, 1978 [includes a table for unequal replication, but only for $\alpha = 0.05$]; Rohlf and Sokal, 1969). In the two-sample case, Hartley's F_{max} is the same as the Folded-F or F' test. The F' test is conducted automatically in the SAS TTEST procedure.

Cochran's Test, where C = the largest variance divided by the sum of the variances, is also simple to calculate by hand, and is somewhat more powerful then Hartley's F_{max} for small, equal sample sizes (Conover et al., 1981). However, tables of critical values of Cochran's C are not available in most statistical texts. Winer (1971) and Dixon and Massey (1983) include a table for Cochran's Test, but the tables are limited to tests with equal sample sizes. Tables of critical values for tests such as Cochran's C and Hartley's F_{max} may also be restricted to one or two α levels (usually 0.05 and 0.01). Because of the limitations of these tables, computer programs are preferred for tests of equality of variances.

Levels of α for tests of equality of variances are provided in Table D-2; these depend upon number of replicates in a treatment (n) and allotment of replicates among treatments (design). Relatively high α's are recommended because the power of the above tests for equality of variances is rather low when n is small. Equality of variances is rejected if the probability associated with the test statistic is less than the appropriate α. If the test for equality of variances is significant even after transformation, the t-test for unequal (separate) variances should be selected rather than the t-test for equal (pooled) variances.

Nonparametric Tests

Tests such as the t-test, which analyze the original or transformed data, and which rely on the properties of the normal distribution, are referred to as parametric tests. Nonparametric tests, which do not require that data be normally distributed, generally analyze the ranks of data, comparing medians rather than means. The median of a sample is the middle or 50th percentile observation when the data are ordered from smallest to largest. In many cases, nonparametric tests can be performed simply by converting the data to ranks or normalized ranks, and then conducting the usual parametric test procedures on the ranks.

Nonparametric tests are useful because of their generality, but may have less statistical power than corresponding parametric tests when the parametric test assumptions are met.

When parametric tests are not appropriate for comparisons because the normality assumption is not met, we recommend converting the data to normalized ranks (rankits). Rankits are simply the z-scores expected for the rank in a normal distribution. Thus, using rankits imposes a normal distribution over all the data, although not

necessarily within each treatment. Rankits can be obtained by ranking the data, then converting the ranks to rankits using the following formula:

$$rankit = z_{[(rank - 0.375) / (N + 0.25)]}, \quad \textbf{(Eq. 7)}$$

where z is the normal deviate and N is the total number of observations. For example, the approximate rankit for the sixth lowest value (rank=6) of 20 would be $z_{[(6 - 0.375)/(20 + 0.25)]}$, which is $z_{0.278}$ or -0.59.

In SAS, normalized ranks or rankits can be provided in PROC RANK with the NORMAL=BLOM option. In SYSTAT and other packages, the ranks must be converted to rankits using the formula above (the conversion is a one-line command). In some programs the conversion may be more difficult to make, especially if functions to provide z-scores for any probability are not available. When rankits cannot easily be calculated, the original data may be converted to ranks.

In comparisons involving only two treatments, there is no real need to test assumptions on the rankits or ranks; simply proceed with a one-tailed t-test for unequal variances using the rankits or ranks.

Statistical Power

For a t-test, the basic formula for calculating the sample size (number of replicate experimental units, n) per treatment necessary to provide a specified power (1-β) to detect a given effect size (d) is:

$$n = 2\,(t_{1-\alpha,\nu} + t_{1-\beta,\nu})^2\,(s^2/d^2), \quad \textbf{(Eq. 8)}$$

where ν = degrees of freedom (df) or $(n_1 + n_2 - 2)$
$t_{1-\alpha,\nu}$ = Student t-value for probability 1-α and ν df
$t_{1-\beta,\nu}$ = Student t-value for probability 1-β and ν df
d = the effect size or difference to be detected.

Recall that β is the probability of committing a Type II error. This formula for n must be solved iteratively, because an initial value of n must be used to determine ν. A new n is then calculated using the initial value, and the process is repeated until n and ν are consistent. The iterative process can be tedious if computer programs are not used. It is easier to use the following approximate formula (from Alldredge, 1987):

$$n = 2\,(z_{1-\alpha} + z_{1-\beta})^2\,(s^2/d^2) + 0.25(z_{1-\alpha}^2), \quad \textbf{(Eq. 9)}$$

where $z_{1-\alpha}$ = normal deviate for 1-α
$z_{1-\beta}$ = normal deviate for 1-β
$0.25(z^2_{1-\alpha})$ = correction term to increase sample size when n is small

Calculated n derived from this formula should be regarded as approximate for $n<5$. Regardless of which formula is used, a fractional n is always rounded up to the next integer.

A useful exercise when sample sizes are fixed because of budget or logistic constraints is to calculate the power of the test to detect a specific effect size (d). In a test comparing 100% elutriate survival with dilution water survival, d is some selected reduction in mean 100% elutriate survival from mean dilution water survival. Eq. 8 can be rearranged and solved for $t_{1-\beta}$ to determine the power:

$$t_{1-\beta,\nu} = \frac{\sqrt{n}d}{\sqrt{2}s} - t_{1-\alpha,\nu} \quad . \qquad \textbf{(Eq. 10)}$$

We then enter a t table at ν df and find the column closest to the value of $t_{1-\beta}$; power ≈ 1-P, where P is the probability for that column. SAS can calculate power more exactly using the PROBT function for $t_{1-\beta}$ and ν df. Note that t-values can be used because both n and ν are known. One can also calculate the difference that can be detected for any given power and sample size:

$$d = (t_{1-\alpha,\nu} + t_{1-\beta,\nu})\sqrt{2s^2/n} \quad . \qquad \textbf{(Eq. 11)}$$

The simplest power to use is 0.50, because then $t_{1-\beta}$=0. Many computer programs will provide this difference, usually referred to as the "minimum significant difference", "least significant difference" or some similar term. The term "average detectable difference" would also be applicable, as this is the difference we expect to be able to detect 50% of the time. In this Appendix, we recommend reporting the minimum significant difference or some other indication of power along with the results of statistical analyses. If power is consistently and regularly reported, investigators will gain an appreciation of the strengths and limitations of various toxicity tests and analyses.

If values are transformed prior to analyses, all power calculations should be done on the transformed scale. In the case of arcsine-transformed survival, a constant effect size d on the percentage or proportion scale will not be constant on the arcsine scale, because the latter scale spreads out high and low values. Therefore, a reference survival must be specified and arcsine-transformed, and the effect size also transformed to a difference on the arcsine scale. For example, suppose we wanted to calculate the power of a t-test to detect a 25% reduction in survival from the reference. A reasonable reference survival (e.g., 90%) would be specified and arcsine-transformed (=1.249). We would also arcsine-transform a 25% reduction (=65% survival or 0.938 after

transformation). The difference *d* would then be 1.249 - 0.938 or 0.311, and that value would be used in power calculations. Experimentation with arcsine-transformed data will rapidly reveal that toxicity tests are more powerful, in terms of the size of differences that can be detected on the original (untransformed) scale, when reference survival is higher. In other words, we are more likely to detect a 25% reduction in survival if reference survival is 90% than if reference survival is 75%. This is precisely what happens in real toxicity tests, which is why the arcsine transformation is used for survival data.

Simple formulae for calculation of sample size or power are not available for the tests of assumptions recommended in this Appendix.

D2.1.1.2 Analysis of Example Data

Table D-3 contains example data from a 96-h water column toxicity test using a dilution water and a dredged-sediment elutriate at four serial dilutions. In this example, control (laboratory) water was also used for dilutions, and no separate control was necessary. In other cases, the dilution water may be receiving water and a separate laboratory control would be required. Analysis of this example data will be conducted using the decision tree in Figure D-1. Numbers in parentheses in the text refer to numbered nodes of the decision tree. The SAS program WATTOX and complete results for water column toxicity test data analyses are provided in Section D4.1; some additional analyses were conducted using SYSTAT programs.

Means (*1*) and SE for the survival data are provided in Table D-3. Overall mean survival in the control (= dilution) water was 98%, indicating that the test was acceptable (*2*). The statistical comparison of 100% elutriate survival and dilution water survival was then conducted because the 100% elutriate survival was at least 10% lower than the dilution water survival (*3*). The next step was to arcsine-transform the survival proportions for the dilution water and 100% elutriate treatments (*4*).

Tests of Assumptions

Following arcsine-transformation, the data were tested for normality (5) to determine whether parametric or nonparametric procedures should be used. Table D-4 provides the results of tests for normality and equality of variances for the example data. The value of Shapiro-Wilk's *W* for the arcsine-transformed data was 0.846, with associated probability (*P*) = 0.051. Because this value of *P* exceeds 0.05 (α level from Table D-2, N=10, balanced design), we conclude that the data do not depart significantly from the normal distribution (*5*), and we now examine the results of the tests for equality of variances (*6*).

Bartlett's Test (from SYSTAT) and F' both indicated that the variances of arcsine-transformed data were not significantly different for the two treatments, with $P>0.10$ (α level from Table D-2, n=5, balanced design). Thus, on the basis of these tests, we would proceed with a *t*-test for equal variances (*7*).

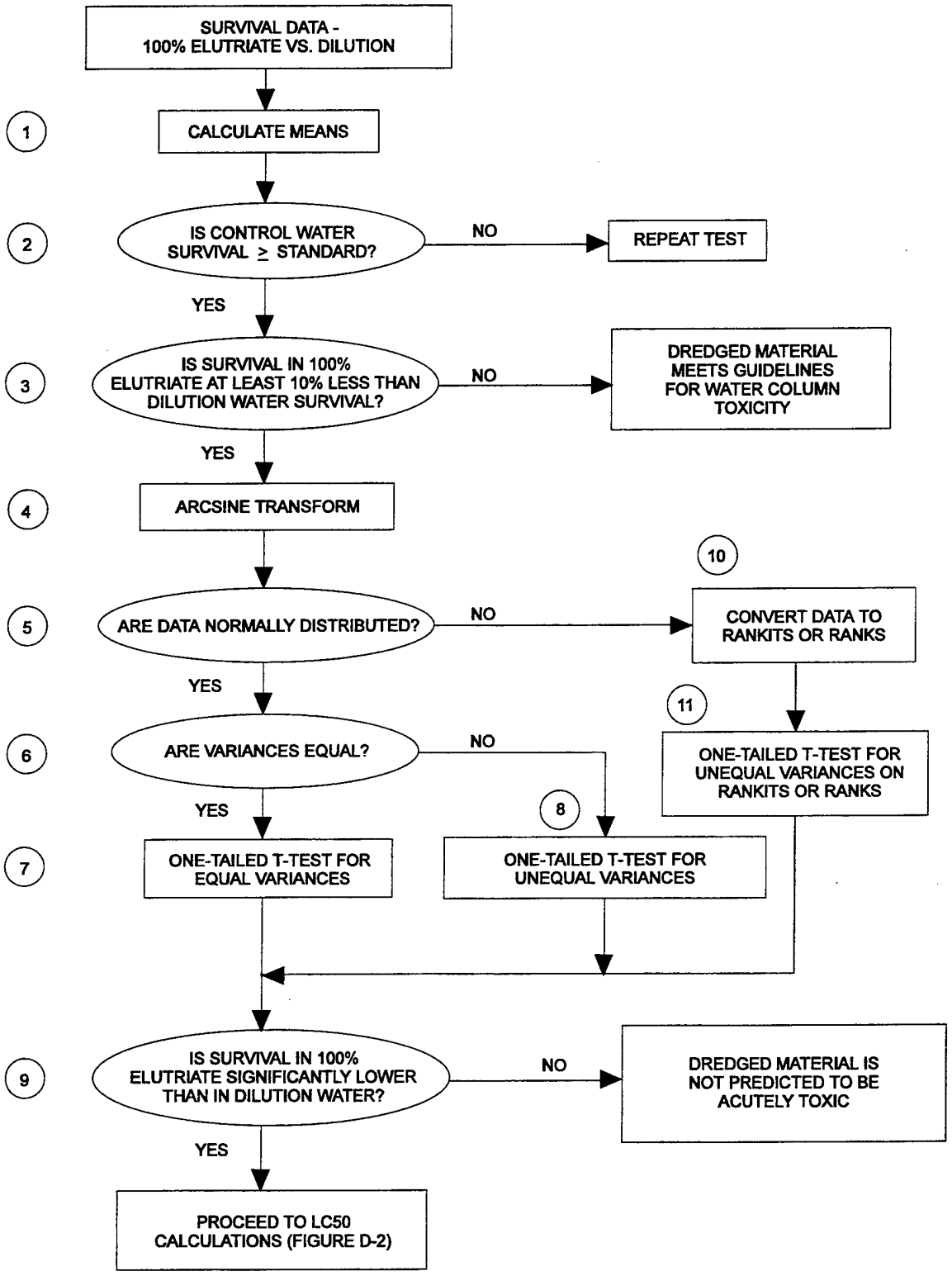


Figure D-1. Water Column Toxicity Test Decision Tree.

Table D-3. Number of Survivors in a Hypothetical Water Column Toxicity Test After 96 h.

Replicate[b]	Treatment[a]				
	Dilution Water[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
Total	**98**	**35**	**46**	**72**	**86**
Mean	19.6 (98%)	7.0 (35%)	9.2 (46%)	14.4 (72%)	17.2 (86%)
SE	0.24	0.71	0.58	1.03	0.37

[a] Percent concentrations of dredged-material elutriate:
100% = 1 part elutriate plus 0 part dilution water
50% = 1 part elutriate plus 1 part dilution water
25% = 1 part elutriate plus 3 parts dilution water
12.5% = 1 part elutriate plus 7 parts dilution water

[b] 20 organisms per replicate at initiation of test

[c] In this example, the dilution water was control (laboratory) water

Table D-4. Tests of Assumptions and Hypothesis Tests on Arcsine-Transformed Water Column Toxicity Test Example Data.

Null Hypothesis: Mean 100% Elutriate Survival Equals Mean Dilution Water Survival[a]				
Test	**Test Statistic**	**Probability *P***	**α**	**Conclusion**
Normality Assumption:				
Shapiro-Wilk's Test	W=0.846	0.051	0.05	do not reject
Equality of Variances Assumption:				
Bartlett's Test	F=0.5	0.47	0.25	do not reject
F' Test	F'=2.18	0.468	0.25	do not reject
Null Hypothesis:				
<u>*t*-Test (equal variances)</u>	<u>t=12.734</u>	<u>≤0.0001</u>	<u>0.05</u>	<u>reject</u>
t-Test (unequal variances)	t=12.734	<0.0001	0.05	reject
t-test on rankits (unequal variances)	t= 4.631	0.0010	0.05	reject

[a] Based on tests of assumptions, appropriate statistical test of null hypothesis is underlined. Other test results are included for illustration only.

Two-sample t-tests

Table D-4 provides the results of *t*-tests for equal (*7*) and unequal variances (*8*). The *t*-test for equal variances indicated that survival in the 100% elutriate was significantly ($P<0.05$) less than in the dilution water (*9*). If the data had been normally distributed with unequal variances, the *t*-test for unequal variances would have been used. With the example data, both test results are the same, but this will not always be the case.

Nonparametric Test

Nonparametric tests would generally not be performed on these data because the sample data did not depart significantly from a normal distribution. However, the data were converted to rankits (10), and a *t*-test for unequal variances (11) was conducted on the rankits (SAS Program WATTOX) for illustrative purposes. The *t*-test indicated that median survival in the 100% elutriate was significantly lower than in the dilution water (Table D-4).

Statistical Power

The difference in survival between the 100% elutriate and the dilution water was so large (63%) that it was easily detected (declared significant) even though there were only five replicates per treatment. The power of a *t*-test to detect such a large decrease in survival (d=0.848 on the arcsine scale) when n=5 and s=0.1055 (also on the arcsine scale) is >0.99. However, it is reasonable to ask if n=5 is adequate for detecting smaller differences. For example, what sample size would be required to provide a ≥0.95 chance ($1-\beta=0.95$; $z_{1-\beta}=1.645$) of detecting a reduction of survival to ≤80%, with $\alpha=0.05$ ($z_{1-\alpha}=1.645$)? In the example data, mean arcsine-transformed dilution water survival was 1.4806 (≈99% survival; back-transformation of means of transformed values will not be the same as means based on original data, although the difference is trivial in this case); the arcsine-transformed value for 80% survival is 1.1071, giving a reduction (d) of 0.3736 on the arcsine scale; and the pooled s was 0.1055. Using Eq. 9:

$$n = 2(1.645 + 1.645)^2 (0.1055^2/0.3736^2) + 0.25(1.645^2) = 2.40$$

Rounding up gives n=3. A more exact iterative computer program (SYSTAT DESIGN) based on *t*-values (Eq. 8) also yields n=3. The sample size required for a 0.95 probability of detecting a reduction in survival to 90% is n=6, again calculated with the iterative program. The minimum significant difference (i.e., the difference we have a 0.50 probability of detecting) when n=5 is $t_{0.95,8}(2s^2/n)^{½}$ or $1.86[2(0.1055^2/5)]^{½} = 0.1241$. Subtracting that from the mean transformed dilution water survival, and back-transforming gives 95.5% survival. In other words, given the example data, the test can be expected to detect a reduction in survival from ≈99% to ≈95-96% approximately half the time.

When dilution water survival is near 100% and variation among replicates is low, as with the example data, a test with n=5 replicates may be too powerful. In many cases, we would declare survival of ≥90% in the 100% elutriate significantly lower than in the dilution water, yet that ≥90% survival would be acceptable for the dilution water. For this reason, if survival in the 100% elutriate is not at least 10% lower than in the dilution

water, the difference should not be considered significant and no statistical tests need be performed. *It is important to remember that a statistically significant difference is not necessarily biologically significant (and vice versa).* If dilution water survival were lower, say 90% instead of 98%, and s remained the same, the t-test would have less power. For example, n=13 would be required to provide a 0.95 probability of detecting a reduction in survival in the 100% elutriate to 80%. Much higher standard deviations can also be expected in many toxicity tests.

The SAS program WATTOX (Section D4.1) provides minimum significant difference and power of a t-test. Power is determined for 10, 20, 30, 40 and 50 percent reductions in true population survival from the mean dilution water survival.

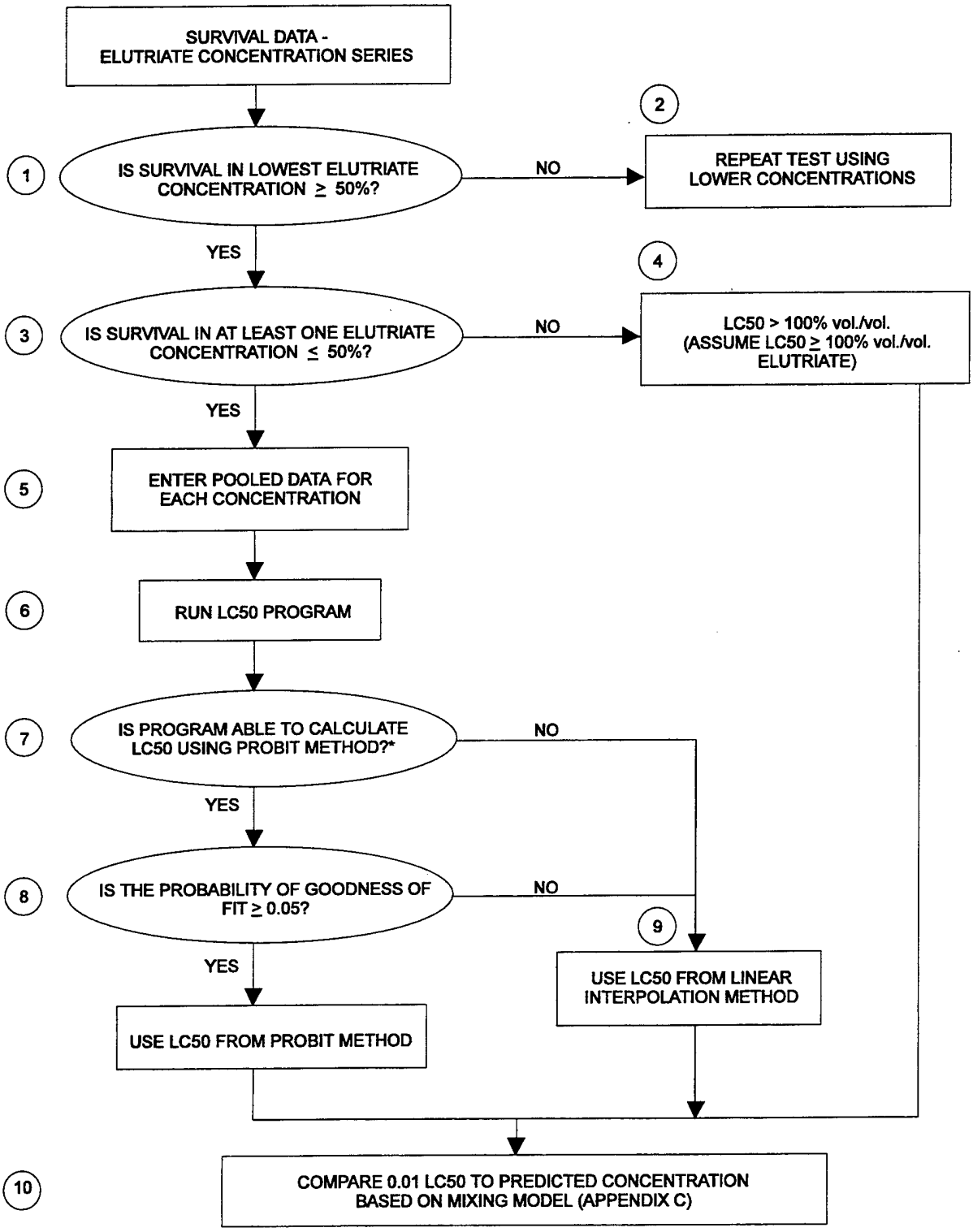
D2.1.2 Calculating Median Lethal Concentration

In Tier III water column toxicity tests, the median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) are calculated when 100% elutriate survival is significantly lower than dilution water survival. The LC_{50} is the concentration lethal to 50% of the test organisms; the EC_{50} is the concentration causing some sublethal effect (e.g., abnormality, immobility) in 50% of the test organisms. The remainder of this section will discuss the LC_{50} but all comments apply equally to EC_{50}. Steps and decisions in the LC_{50} determination are shown in the decision tree in Figure D-2. Numbers in parentheses in the text refer to numbered nodes of the decision tree.

Ideally, data for at least five elutriate concentrations should be available to calculate an LC_{50}, although most methods described below can be used for fewer concentrations. The control or dilution water survival is not included. Survival in the lowest elutriate concentration must be at least 50% (*1*); otherwise the test must be repeated using lower concentrations (*2*). An LC_{50} should not be calculated unless at least 50% of the test organisms die in at least one of the serial dilutions (*3*). If there are no mortalities greater than 50%, then the LC_{50} is assumed to be ≥100% elutriate (*4*).

If the conditions in (*1*) and (*3*) are met, then replicate mortality data for each concentration are pooled (*5*) for calculation of LC_{50} (*6*). The Probit method (*7*) can be used if the data meet the requirements of the Probit method listed below and fit the probit model (*8*). The Trimmed Spearman-Karber (TSK) and Logistic methods (described below) are acceptable substitutes for the Probit method, provided that the data meet the requirements of these alternative methods. If the data do not meet the requirements of the Probit method or alternatives, then the Linear Interpolation method should be used (*9*). When an LC_{50} value has been determined, 1% of that value is entered into the mixing model (*10*) provided in Appendix C for mixing zone evaluation.

Calculation of LC_{50} values is also recommended for reference toxicant tests to determine the relative health of the organisms used in toxicity and bioaccumulation testing (Section 13.3.17.2).



* Trimmed Spearman-Karber and logistic methods are acceptable substitutes for Probit method.

Figure D-2. LC_{50} Decision Tree.

D2.1.2.1 Methods For Calculating LC_{50}

Stephan (1977) and Gelber et al. (1985) provide careful reviews of LC_{50} estimation procedures. In addition, USEPA (1985) discusses in detail the mechanics of calculating LC_{50} using current methods and contains, as an appendix, computer programs for each statistical method. The most commonly used methods are the Probit, Trimmed Spearman-Karber (TSK) and Linear Interpolation. This Appendix recommends use of the Probit, TSK or Logistic methods if the data are appropriate; otherwise the Linear Interpolation method may be used (Figure D-2). In general, results from different methods should be similar. Programs commonly used to calculate LC_{50} are PROBIT, developed for and available from the USEPA (Environmental Monitoring and Support Laboratory, Cincinnati, OH), and several programs developed by Dr. C.E. Stephan of the USEPA Environmental Research Laboratory in Duluth, Minnesota. Procedures in statistical packages such as SAS or SYSTAT may not be easily adaptable for routine calculations of LC_{50}, and specialized packages are generally preferred. This Appendix does not include SAS programs for LC_{50}.

Probit

The Probit method is based on regression of the probit of mortality on the log of concentration. A probit is the same as a z-score; for example, the Probit corresponding to 70% mortality is $z_{0.70}$ or ≈ 0.52. The LC_{50} is calculated from the regression, and is the concentration associated with $z=0$ (mortality = 50%). The Probit method can be used whenever the following conditions are met:

- there are at least two concentrations with partial mortality (i.e., >0 and <100%)
- the data points fit the probit regression line reasonably well.

The first condition is necessary because the regression line is estimated from the partial mortalities. The second condition, called goodness-of-fit, can be tested by the χ^2 statistic, which is a measure of the distance of the data points from the regression line. A low χ^2 indicates a good fit. By convention, the fit is considered adequate if the P-value for χ^2 is >0.05 (in other words, goodness-of-fit is rejected if $P \leq 0.05$). Programs such as PROBIT will only provide χ^2, in which case χ^2 should be compared against tabled values with k - 2 df, where k is the number of partial mortalities. If there are only two partial mortalities (k=2), then there are 0 df, and the goodness-of-fit cannot be tested (i.e., a line between two points is always a perfect fit). When there are only two partial mortalities, the LC_{50} is identical to the LC_{50} which would be calculated by Linear Interpolation (see below) with mortality expressed on a probit scale. Goodness-of-fit can also be assessed by eye, if the data are plotted on log-probit paper, or if the computer program provides a plot.

Linear Interpolation Method

The Linear Interpolation method should be used when:

- there are 0 or 1 partial mortalities

- the data do not fit the Probit (or Logistic) models

The Linear Interpolation method should also be used when LC_{50}s are calculated and compared over an extended time series (i.e., for tracking reference toxicant results), because inevitably, one or more data sets will fail to meet the requirements for the Probit, TSK or Logistic methods. Linear Interpolation may also be used if programs for the other methods are unavailable, but we strongly recommend that investigators have programs available for one or more of the other methods.

The Linear Interpolation method calculates an LC_{50} by interpolation between the two concentrations with mortality nearest to, and on either side of 50%. The interpolation is made on a log concentration scale, using the following formula:

$$LC_{50} = antilog \frac{(50 - M_L)(\log C_U) + (M_U - 50)(\log C_L)}{M_U - M_L} , \qquad \textbf{(Eq. 11)}$$

where C_L = concentration with mortality nearest to and below 50%
C_U = concentration with mortality nearest to and above 50%
M_L = % mortality at C_L
M_U = % mortality at C_U.

If there are no partial mortalities, the formula simplifies to:

$$LC_{50} = \sqrt{(C_U)(C_L)} .$$

For the example data given in Table D-3, C_L=25% elutriate (log=1.398); M_L=28% mortality; C_U=50% elutriate (log=1.699); and M_U=54% mortality. Therefore:

$$LC_{50} = antilog \frac{(50 - 28)(1.699) + (54 - 50)(1.398)}{54 - 28} ,$$

or 44.9%.

The formula and example given above express mortality on an arithmetic (untransformed) scale. Some computer programs or investigators may use arcsine-transformed mortalities (Stephan, 1977; see Section D.2.1.1.1 Tests of Assumptions). One could also express mortality on a probit or logit scale, if there were one partial mortality on each side of 50%. In those cases, the Linear Interpolation should produce the same LC_{50} estimate as the Probit or Logistic methods. In this manual, we recommend the use of untransformed mortality for simplicity and consistency. However, LC_{50} estimates using other scales can easily be calculated for comparison.

Trimmed Spearman-Karber (TSK) Method

The TSK method is a nonparametric method that can be calculated by hand using the procedure in Gelber et al. (1985). The calculations can be tedious, especially for processing large numbers of tests, and computer programs are usually used. The method is labelled "trimmed" because extreme values (mortality much higher or lower than 50%) are "trimmed" or removed prior to calculation of the LC_{50}. Thus, the LC_{50} is calculated using points near 50% mortality, which may produce a more robust estimate. The TSK method can be used in many cases where the Probit method is unsuitable. Access to appropriate computer programs, and difficulties in deciding what values to trim are probably the major factors limiting widespread use of the TSK method. Investigators with access to reliable programs should not hesitate to use the TSK method whenever there are two or more partial mortalities. Information concerning TSK computer programs may be obtained from the USEPA Environmental Research Laboratories in Athens, GA, or Duluth, MN, or CSC/USEPA, Cincinnati, OH.

Logistic Method

The Logistic method is similar to the Probit method except that mortalities are converted to logits rather than probits. A logit is log $[M/(100 - M)]$, where M is % mortality. The LC_{50} is derived from a regression of logits on log concentration. As with the Probit method, the Logistic method can be used whenever there are two or more partial mortalities, and the data fit the regression line. Logistic regression is not commonly used in aquatic toxicology only because Probit programs are more available, but the two methods are equally acceptable. Logistic regression programs in SAS and SYSTAT are designed for complex analyses and comparisons of logistic regressions, and may be inconvenient to use for simple and routine calculations of LC_{50} for single tests.

D2.1.2.2 Analysis of Example Data

Table D-5 provides LC_{50} estimates calculated by several different methods using the example data in Table D-3. In all cases, the data from the five replicates for each concentration were pooled, and entered as the number responding (dying) out of 100. *Because pooling over replicates ignores any additional variance in survival among replicates (i.e., beyond the expected error from sampling the binomial distribution), the confidence limits provided by the programs may not be accurate and should not be reported or used.* Because the LC_{50} is required only for use in the mixing model (Appendix C), confidence limits are not needed.

Table D-5. Calculated LC_{50} Values for Example Water Column Toxicity Test Data.

Method	LC_{50} Estimate (% v/v)
Probit	52.6
Linear Interpolation - untransformed mortality - arcsine-transformed mortality	 44.9 45.1
Trimmed Spearman-Karber	48.4
Logistic	52.6

The Probit LC_{50} was calculated with the EPA PROBIT program, and was almost identical to the Logistic LC_{50} calculated using the SYSTAT LOGISTIC program. The χ^2 goodness-of-fit for the Probit line was 1.756, indicating a good fit ($P>0.05$ with 4 - 2 = 2 df), which could be verified by examining the plot provided (Figure D-3). The LC_{50} estimated by Linear Interpolation, with untransformed mortality, was almost identical to the LC_{50} calculated using arcsine-transformed mortality. The TSK LC_{50} was calculated using a program modified from an original program described in Hamilton et al. (1977), and was intermediate between the Linear Interpolation and regression (Probit and Logistic) estimates.

The various estimates in Table D-5 differed by up to 7.7% elutriate, which is not unusual or alarming. The Probit or Logistic LC_{50} would be the preferred estimate, because the regression lines fit the data well, and the regression methods use more of the data in such cases. However, any of the estimates would be adequate for use in the mixing model in Appendix C, because the imprecision and uncertainty involved in the model calculations and estimates are undoubtedly far greater than the differences among the LC_{50} estimates.

D2.2 Tier III Benthic Toxicity Tests

The objective of Tier III benthic toxicity tests is to determine if sediments taken from a potential dredge site are significantly more toxic than a reference sediment. The test procedure is described in Section 11.2. The statistical analysis recommended below assumes that individual dredge sites are relatively large, and that a decision about potential sediment toxicity, and subsequently about disposal options, will be made independently for each site. If only one dredge site is tested, and compared to a reference sediment, statistical analysis is the same as that given in Section D2.1.1 for comparison of 100% elutriate and dilution water (Figure D-1 and SAS program WATTOX in Section D4.1). However, in many cases, more than one dredge site is tested simultaneously with one reference sediment. In those cases, recommended statistical methods will differ from the two-sample case. Methods for comparison of more than one dredged sediment with a reference sediment are described below, and computer procedures are given in SAS program BENTOX (Section D4.2).

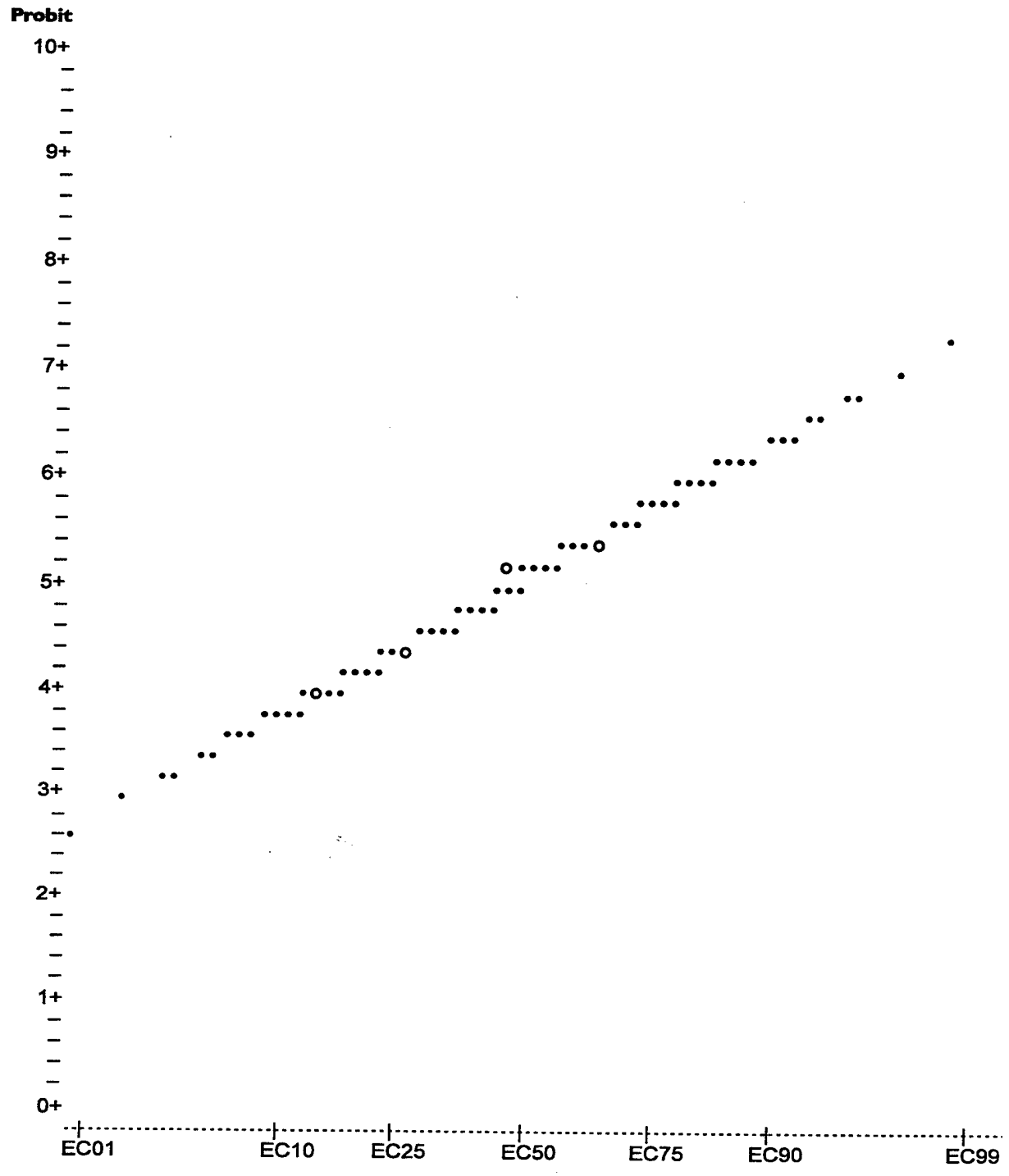


Figure D-3. Probit Plot of Water Column Toxicity Test Example Data.

D2.2.1 Methods

Fisher's Least Significant Difference (LSD)

Fisher's Least Significant Difference (LSD) is the appropriate parametric statistical test for assessing differences in survival or other response when more than two means are being compared. This *a posteriori* multiple comparison technique is discussed in many statistical texts, e.g., Steel and Torrie (1980); SAS Institute, Inc. (1988b); Snedecor and Cochran (1989); and Wilkinson (1990). The LSD controls the pairwise Type I error rate rather than the experimentwise Type I error rate. This means that when the test assumptions are met, the Type I error rate for each comparison is held to the preset α even though the overall Type I error rate for all comparisons (i.e., experimentwise error rate) may be higher. A test that controls the pairwise error rate is appropriate because disposal decisions are to be made independently for each dredge site regardless of how many sites are compared to the same reference. The LSD replaces the previously recommended Dunnett's test, which is not appropriate because it controls experimentwise error rate.

The LSD is usually performed in conjunction with analysis of variance (ANOVA), and only if the data meet the assumptions of normality and equal variances. The ANOVA is conducted primarily to provide the mean square error (*MSE*), which is an estimate of the pooled variance across all treatments. The ANOVA *F*-statistic and its associated probability are ignored in this application.

The test statistic for the LSD is *t*, calculated in much the same way as for a *t*-test:

$$t = (\bar{x}_1 - \bar{x}_2) \,/\, \sqrt{MSE\ (1/n_1 + 1/n_2)} \qquad \textbf{(Eq. 13)}$$

This *t*-statistic is compared against the distribution of Student's *t* with $N - k$ degrees of freedom, where N is the total number of observations (Σn) and k is the number of treatments including the reference. A *t*-statistic is computed for each possible pair of treatments in the analysis.

The *MSE* can be calculated as:

$$MSE = \Sigma[s_i^2\ (n_i - 1)] \,/\, \Sigma(n_i - 1) \quad , \qquad \textbf{(Eq. 14)}$$

where s_i^2 and n_i are the variance and number of replicates for the ith treatment. The term $\Sigma(n_i - 1)$ is equivalent to $N - k$.

If sample sizes are equal, then:

$$MSE\ (1/n_1 + 1/n_2) = 2MSE/n \quad . \qquad \textbf{(Eq. 15)}$$

The major advantage of using the LSD as opposed to conducting individual two-sample *t*-tests comparing each dredged sediment to the reference is that the *MSE* is a better estimate of the true population variance than the pooled variance calculated from only two samples. Consequently, the LSD test is more powerful, as reflected in the greater df for the calculated *t*. It also follows that a pooled variance should only be calculated, and the LSD test conducted, if the variances for the treatments are not significantly different.

Tests of Assumptions

The Shapiro-Wilk's Test described in Section D2.1.1.1 can also be used to test for normality when more than two treatments are compared. If the data are not normally distributed, even after an appropriate transformation, then nonparametric tests should be used (see Nonparametric Tests below).

Bartlett's Test, Levene's Test, F_{max}, or Cochran's Test can be used to test for equality of variances. If there are more than two samples, then F_{max} is equal to the largest variance divided by the smallest variance. If variances are significantly unequal, even after transformation, then each dredged sediment should be compared with the reference using two-sample *t*-tests.

Nonparametric Tests

When parametric tests are not appropriate for multiple comparisons because the normality assumption is violated, the data should be converted to rankits, and the rankits should be tested for normality and equality of variances. If these assumptions are not violated, an LSD is then performed on the rankits (Conover, 1980, refers to this as van der Waerden's Test). Tests performed on rankits are robust to departures from normality, and can still be used when the normality assumption is violated. Rankits will rarely fail tests for normality, partly because a normal distribution is imposed over the entire data set. The rankit data may fail the test for equality of variances, but then *t*-tests can be conducted for each pair of treatments to be compared. If rankit-transformed data fail normality tests, it is probably safest to use the *t*-tests for unequal variances, as some tests for equality of variance are not robust when data are non-normal.

When rankits cannot be easily calculated, the original data may be converted to ranks (using SAS PROC RANK, for example). Equality of variances should be tested after the data are ranked. There is a common misconception that nonparametric tests can be used when variances are not equal as well as when data are not normally distributed. However, nonparametric tests are not very robust if the variances of the ranks are not similar among treatments. Bartlett's Test should not be used to test equality of variances of ranks, as ranks will follow a uniform, rather than a normal distribution, and Bartlett's Test is unduly sensitive to non-normality. Other tests discussed in Section D2.1.1.1 Tests for Equality of Variances may be used on ranks; there are also nonparametric tests for equality of variances provided in Conover (1980).

If the variances of the ranks are not significantly different, the Conover *T*-Test (Conover, 1980) should be performed. This test can most easily be conducted by performing an LSD on the ranks. If the variances of ranks are significantly unequal, a one-tailed *t*-test for unequal variances should be performed (using ranks) for each pair of treatments to be compared.

Statistical Power

Power calculations for the LSD are the same as for the *t*-test (see Eq. 8), except that the degrees of freedom for $t_{1-\alpha}$ and $t_{1-\beta}$ are $N - k$, and *MSE* replaces s^2:

$$n = 2\,(t_{1-\alpha,\nu} + t_{1-\beta,\nu})^2\,(MSE/d^2)\,, \qquad \textbf{(Eq. 16)}$$

If the *z*-approximation (Eq. 9 with *MSE* replacing s^2) is used to calculate samples size, the result will be a slight overestimate, although the overestimation is rarely of practical importance. Finally, the minimum significant difference should be reported for LSD tests. Note that the test is named the Least Significant Difference because another way to conduct the test is to compare the observed differences to the minimum significant difference.

If an increase in power (1-β) is desired, because variance is high or sample size low, one effective method of increasing power is to increase the number of reference replicates rather than increase the sample size for each treatment. It is even possible to increase power without increasing overall sample size by increasing sample size for the reference, and decreasing sample size for the dredged sediments. The optimal apportionment of replicates is to make the sample size for the reference $\sqrt{k}$ times the sample size for the other sediments (Dunnett, 1955). Increasing sample size for the reference sediment is effective because the reference is involved in every comparison, whereas the dredged sediments are involved in only one comparison each.

D2.2.2 Analyses of Example Data

Table D-6 presents survival data from a hypothetical benthic toxicity test comparing survival from three dredged sediments with reference sediment survival. The example data are used to illustrate the steps in benthic toxicity data analysis, with numbers in parentheses in the text referring to numbered nodes in the decision tree (Figures D-4A,B). In this example, survival in the control (data not shown) was ≥90%, indicating the acceptability of the test (Figure D-4A,*1*). Mean survival in all dredged sediments was more than 10% below mean survival in the reference sediment, indicating that the significance of the reductions should be tested statistically (*2*). All data were arcsine-transformed prior to analyses (*3*). Data were analyzed using SAS program BENTOX (Section D4.2), and results for the analyses are given in Section D4.2.2.

Table D-6. Number of Survivors in a Hypothetical Benthic Toxicity Test.

Replicate[a]	Treatment			
	Reference	**Sediment 1**	**Sediment 2**	**Sediment 3**
1	20	17	15	17
2	20	16	16	12
3	19	18	13	10
4	19	17	17	16
5	20	15	11	13
Total	**98**	**83**	**72**	**68**
Mean	19.6 (98%)	16.6 (83%)	14.4 (72%)	13.6 (68%)
SE	0.24	0.51	1.08	1.29

[a] 20 organisms per replicate at initiation of test

Tests of Assumptions

Following arcsine-transformation, the data were tested for normality (*4*) to determine whether parametric (Figure D-4A) or nonparametric (Figure D-4B) procedures should be used. Results of tests for normality (*4*) and equality of variances (*5*) are provided in Table D-7. The *P*-value for the Shapiro-Wilk's Test was 0.32, indicating no significant departure from normality because *P* exceeds 0.01 (α level in Table D-2 for N=20, balanced data). Bartlett's Test, Levene's Test, and F_{max} all indicated that variances were not significantly different among groups, as all *P*-values were >0.10 (α level in Table D-2 for n=5, balanced data). Note that these three tests were included for the sake of comparison, but generally only one of them would be conducted. Because the data are normally distributed and variances are not significantly different, the LSD is the most appropriate test for comparing each dredged sediment to the reference (*6*).

Parametric Tests

Relevant results from the LSD test are provided in Table D-7 (note that LSD results are given separately for each dredged sediment-reference sediment comparison, but only one LSD test is actually performed, comparing each pair of sediments simultaneously). The *P*-values for the LSD comparisons of each sediment with the reference were all much less than 0.05; thus, we conclude that survival in each of the dredged sediments was significantly less than reference sediment survival (*7*). SAS output for the LSD test (Section D4.2.2) does not provide *t*-values and probabilities for the individual comparisons, and it is not necessary to calculate these.

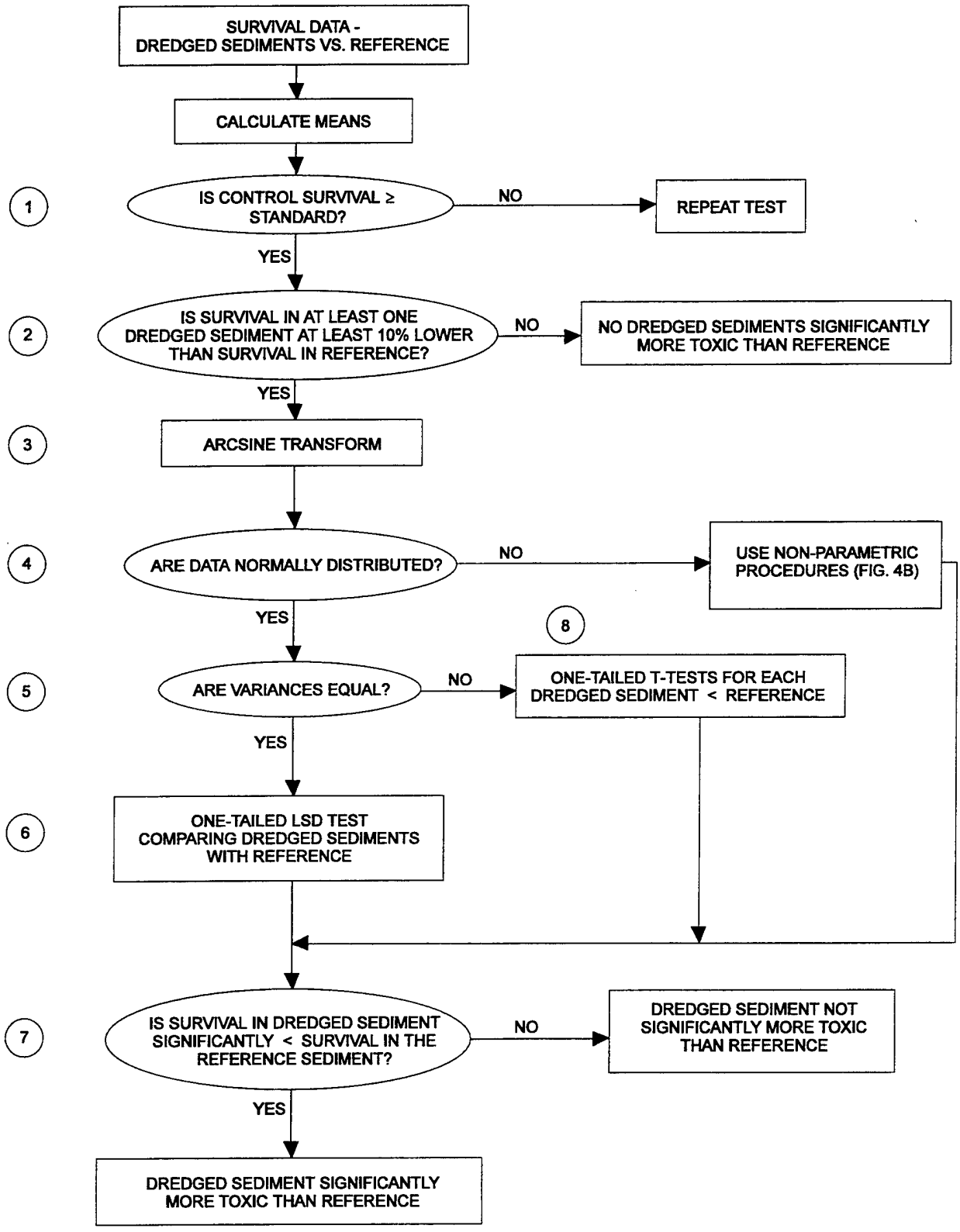


Figure D-4A. Benthic Toxicity Test Decision Tree (Parametric Tests).

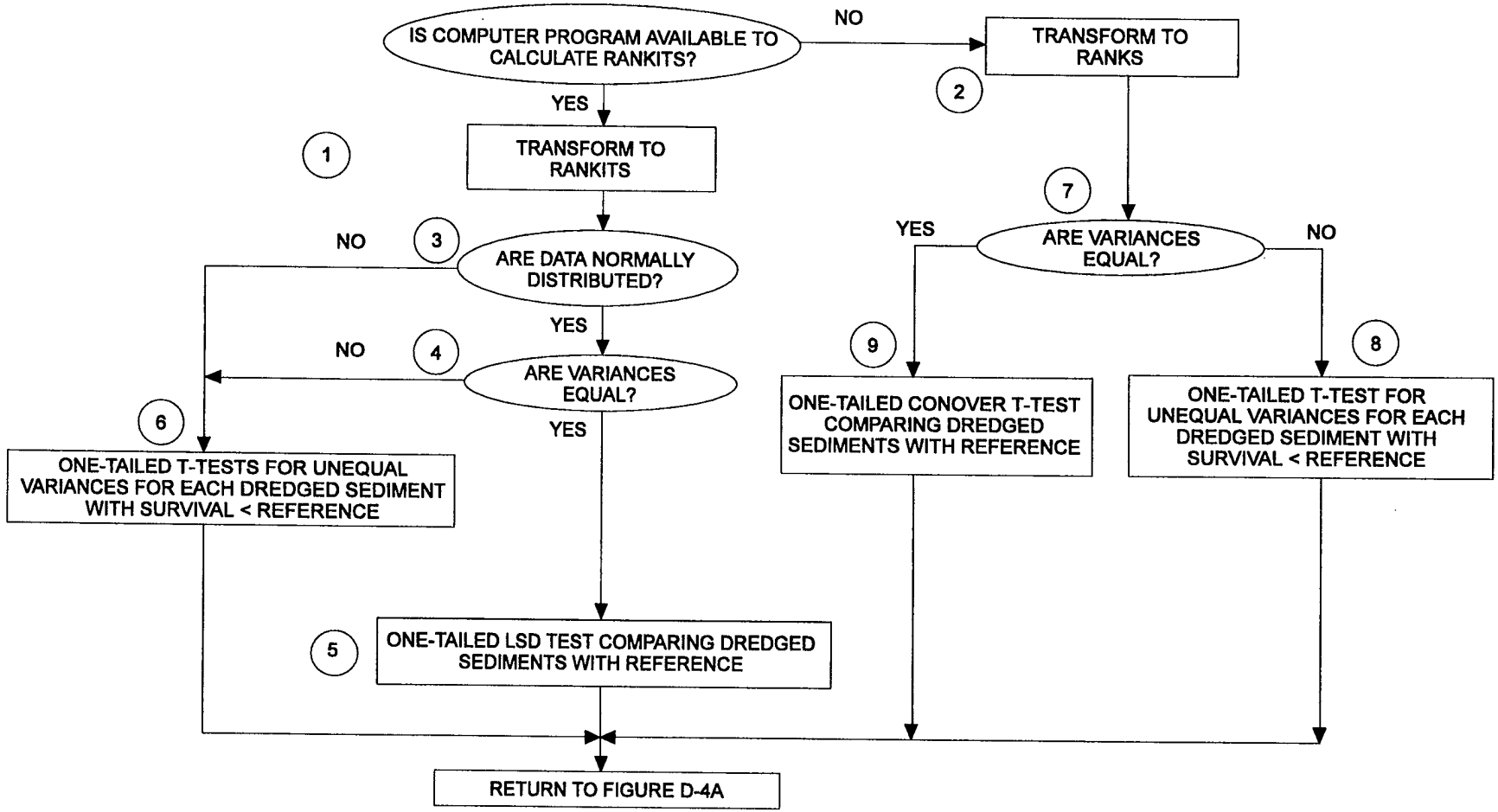


Figure D-4B. Benthic Toxicity Test Decision Tree (Nonparametric Tests).

Table D-7. Tests of Assumptions and Parametric Tests of Hypotheses on Arcsine-Transformed Benthic Toxicity Test Example Data.

Null Hypothesis: Mean Dredged Sediment Survival Equals Mean Reference Sediment Survival[a]				
Test	**Test Statistic**	**Probability *P***	**α**	**Conclusion**
Normality Assumption:				
Shapiro-Wilk's Test	W=0.946	0.322	0.01	do not reject
Equality of Variances Assumption:				
Bartlett's Test	F=0.6	0.61	0.10	do not reject
Levene's Test	F=1.74	0.199	0.10	do not reject
F_{max} Test	F_{max}=4.4	>0.25	0.10	do not reject
Null Hypotheses:				
Sediment 1 = Reference				
LSD Test	t=4.11	0.0017	0.05	reject
t-Test (unequal variances)	t=5.09	0.0009	0.05	reject
Sediment 2 = Reference				
LSD Test	t=5.73	0.0002	0.05	reject
t-Test (unequal variances)	t=5.63	0.0003	0.05	reject
Sediment 3 = Reference				
LSD Test	t=6.25	0.0001	0.05	reject
t-Test (unequal variances)	t=5.57	0.0004	0.05	reject

[a] Based on tests of assumptions, appropriate statistical tests of null hypotheses are underlined. Other test results are included for illustration only.

SAS indicates significant differences by using different letters under the "T Grouping" column. Mean reference survival was highest (A); mean survivals for sediments 1 (B) and 2 (BC) were significantly less than reference but not different from each other, and sediment 3 mean survival (C) was significantly lower than reference and sediment 1 but not sediment 2.

If the variances had been unequal, survival data would have been compared using t-tests (*8*). These results are included in Table D-7 for illustration. Again, the P-values indicate that all dredged sediment survivals were significantly less than reference sediment survival. Note that these P-values are one-half those given in the output from SAS program BENTOX in Section D4.2.2, because the SAS TTEST procedure returns two-tailed, rather than one-tailed probabilities.

Nonparametric Tests

Although the arcsine-transformed example data did not violate parametric hypothesis testing assumptions, nonparametric tests were performed to illustrate the steps in the nonparametric decision tree (Figure D-4B). The example data were converted using both rankits (*1*) and ranks (*2*), and the appropriate tests of assumptions were conducted (Table D-8). The rankits passed both the normality (*3*) and equality of variances (*4*) tests, so the next

step would be the LSD on rankits (*5*). Had either of these assumptions been violated, *t*-tests for unequal variances would have been performed on the rankits (*6*). If the ranks had failed the Levene's Test for equality of variances (*7*), *t*-tests for unequal variances would have been performed on the ranks (*8*), rather than the Conover *T*-Test (*9*). Results for all of these nonparametric hypothesis tests are shown in Table D-8. SAS Program BENTOX does not perform Levene's Test on ranks, the Conover *T*-Test, or 2-sample *t*-tests on ranks, as SAS can easily calculate rankits, and ranks-based tests would not be needed. The *P*-values for the nonparametric hypothesis tests in Table D-8 were in most cases slightly greater than those for the parametric tests, suggesting slightly lower power for the nonparametric tests. Nevertheless, all tests indicated that survival was significantly reduced in the dredged sediments compared to reference sediment survival. These results could easily have been predicted prior to analyses, because survival in the dredged sediment samples did not overlap with survival in the reference sediment samples (Table D-6).

Table D-8. Tests of Assumptions and Nonparametric Hypothesis Tests on Benthic Toxicity Test Example Data Converted to Rankits and Ranks.

Null Hypothesis: Median Dredged Sediment Survival Equals Median Reference Sediment Survival				
Test	**Test Statistic**	**Probability *P***	α	**Conclusion**
Normality Assumption:				
Shapiro-Wilk's Test (rankits)	W=0.982	0.940	0.01	do not reject
Equality of Variances Assumption:				
Levene's Test (rankits)	F=1.18	0.349	0.10	do not reject
Levene's Test (ranks)	F=2.25	0.122	0.10	do not reject
Null Hypotheses:				
Sediment 1 = Reference				
LSD Test (rankits)	t=3.05	0.0079	0.05	reject
t-Test (rankits, unequal variances)	t=4.57	0.0011	0.05	reject
Conover *T*-Test (ranks)	t=3.04	0.0080	0.05	reject
t-Test (ranks, unequal variances)	t=4.27	0.0036	0.05	reject
Sediment 2 = Reference				
LSD Test (rankits)	t=4.71	0.0008	0.05	reject
t-Test (rankits, unequal variances)	t=5.44	0.0007	0.05	reject
Conover *T*-Test (ranks)	t=4.90	0.0006	0.05	reject
t-Test (ranks, unequal variances)	t=5.80	0.0012	0.05	reject
Sediment 3 = Reference				
LSD Test (rankits)	t=5.28	0.0004	0.05	reject
t-Test (rankits, unequal variances)	t=4.91	0.0019	0.05	reject
Conover *T*-Test (ranks)	t=5.30	0.0004	0.05	reject
t-Test (ranks, unequal variances)	t=5.51	0.0018	0.05	reject

Statistical Power

From Eq. 11, the minimum significant difference (d_{min}, when $t_{1-\beta}$=0) for the parametric LSD test was:

$$d_{min} = (t_{1-\alpha,\nu})\sqrt{2MSE/n} \quad \textbf{(Eq. 17)}$$

= 1.746[2(0.01618)/5]^{½} = 0.1405, where ν=16 df. Subtracting 0.1405 from the mean arcsine-transformed survival in the reference (1.481), and back-transforming gives 95%. That is, any survival less than 95% measured in a sample would be significantly lower than in the reference, and we would have a 0.50 probability of detecting a reduction in survival in any case where true population survival was 95%. Modifying Eq. 10, the probability (power or 1-β) of detecting a difference if true population survival in a dredged sediment is <90% can be determined by:

$$t_{1-\beta,\nu} = d\sqrt{n/2MSE} - t_{1-\alpha,\nu} \quad \textbf{(Eq. 18)}$$

= (1.481 - 1.249) [5/2(0.01618)]^{½} - 1.746 = 1.138. Using the SAS PROBT function to determine 1-β for t=1.138 with 16 df, power = 0.86. As with the water column toxicity test example data, the level of replication for the benthic toxicity example data is adequate to detect any reductions in survival that would be considered biologically significant. Investigators can expect lower reference survival and/or greater variance, and consequently less power, in real toxicity tests.

Suppose that we required an increase in power, but could not afford to add any more replicates. The optimal solution, assuming that variance could not be reduced by improving laboratory practices, would be to use 8 replicates for the reference, and 4 for each of the dredged sediments. The overall sample size remains 20. Note that a ratio of reference:dredged sediment replicates of 8:4 (2:1) is approximately equal to the optimal ratio of $\sqrt{k}$:1 or 1.73:1 (k=3 with 3 dredged sediments). Assuming that MSE=0.01618, as above, the minimum significant difference for an LSD test, again with 16 df, would be:

$$d_{min} = (t_{1-\alpha,\nu})\sqrt{MSE(1/n_1 + 1/n_2)} \quad \textbf{(Eq. 19)}$$

= 1.746[0.01618(1/4 + 1/8)]^{½} = 0.1360. This value is lower, although by <5%, than the minimum significant difference of 0.1405 for equal sample sizes of 5. The increase in power using the optimal ratio of reference:dredged sediment replicates will be greater when k is greater (more sediments tested).

SAS program BENTOX (Section D4.2) provides power calculations for the LSD test when true population survival from a dredged sediment is 10, 20, 30, 40 and 50 percent lower than mean reference sediment survival.

D3.0 BIOACCUMULATION

Bioaccumulation tests described in Section 12 are applied to determine whether an organism's exposure to the dredged material is likely to cause an elevation of contaminants in its tissues, i.e., bioaccumulation. Bioaccumulation tests may be conducted in the laboratory or in the field. Data analysis for these tests uses statistical procedures that have already been described for benthic toxicity test data analysis. These procedures are illustrated with example data in the following sections.

Statistical procedures for bioaccumulation data analysis in this Appendix cannot be applied directly in the common situation where some contaminant concentrations are reported only as less than some numerical detection limit (DL). The actual concentrations of these "censored" data (hereafter referred to as nondetects) are unknown and are presumed to fall between zero and the DL. Whenever possible, laboratories that analyze contaminant residues should be encouraged to report observed concentrations below DL (Porter et al., 1988), even though the precision of these measurements is less than that of above-DL measurements. When below-DL concentrations (sometimes called "J-values") are reported, they should be used as legitimate data in statistical comparisons. On the other hand, when bioaccumulation samples include nondetects, the unknown values must be replaced using a censored data method prior to statistical analysis. Recommended censored data methods are discussed in Sections D3.1.1.1 and D3.1.2.1.

D3.1 Tier III Single-Time Point Laboratory Bioaccumulation Study

The Tier III single-time point laboratory bioaccumulation test produces tissue concentration measurements for each contaminant of concern. Table D-9 presents example results for one contaminant from a hypothetical laboratory test. Chemical analysis of the tissue samples from each replicate shows that concentrations of the example contaminant varied among and within sediments. Two types of analyses may be performed on these data:

- comparisons between each dredged sediment and the reference, and
- comparisons with an action level when applicable.

Although Section 6.3 stipulates that applicable comparisons with an action level be conducted first, the statistical analysis can be performed more efficiently if comparisons with the reference are done first. Computer procedures for statistical analysis of single-time point bioaccumulation data are given in SAS program BIOACC (Section D4.3).

Table D-9. Results from a Hypothetical Single-Time Point Bioaccumulation Test, Showing Contaminant Concentrations (µg/g) in Tissues of Animals Exposed to Different Treatments.

Replicate	Treatment			
	Reference	**Sediment 1**	**Sediment 2**	**Sediment 3**
1	0.06	0.16	0.24	0.13
2	0.05	0.19	0.10	0.05
3	0.05	0.18	0.13	0.17
4	0.08	0.22	0.18	0.08
5	0.09	0.31	0.30	0.22
Mean	0.066	0.212	0.190	0.130
SE	0.008	0.026	0.036	0.030

D3.1.1 Comparisons with a Reference Sediment

Analysis of the example data follows the decision tree steps in Figures D-5A and 5B, with numbers in parentheses in the text referring to numbered nodes of the decision trees. The objective of this type of analysis is to determine whether organisms exposed to the dredged sediments accumulate greater tissue contaminant levels than organisms exposed to the reference sediment. One-sided tests are appropriate because there is little concern if bioaccumulation from a dredged sediment is less than bioaccumulation from the reference sediment. If mean tissue concentrations of contaminants of concern in organisms exposed to a dredged sediment are less than or equal to those of organisms exposed to the reference sediment (*1*), the dredged sediment meets the guidelines (Section 6.3), and no statistical analysis is required.

If only one dredged sediment is compared to the reference, then the procedures described in Section D2.1.1.1 (tests of assumptions followed by a *t*-test using a transformation or rankits if necessary) for comparing two samples are used. If more than one sediment is compared to the reference, then the procedures described in Section D2.2.1 (tests of assumptions followed by LSD, *t*-tests, or nonparametric equivalents) are used. Because contaminant concentration data are not easily expressed as proportions, the arcsine transformation is not appropriate. The raw data are analyzed first and, if necessary, a logarithmic (either natural or base 10) transformation may be employed. Although other transformations (such as square root) are possible, we recommend the log transformation because contaminant concentration data often follow a lognormal distribution. As always, tests of assumptions must be rerun on the data following transformation. If the transformed data violate the normality assumption, then data are converted to rankits (or ranks) and the assumptions are retested.

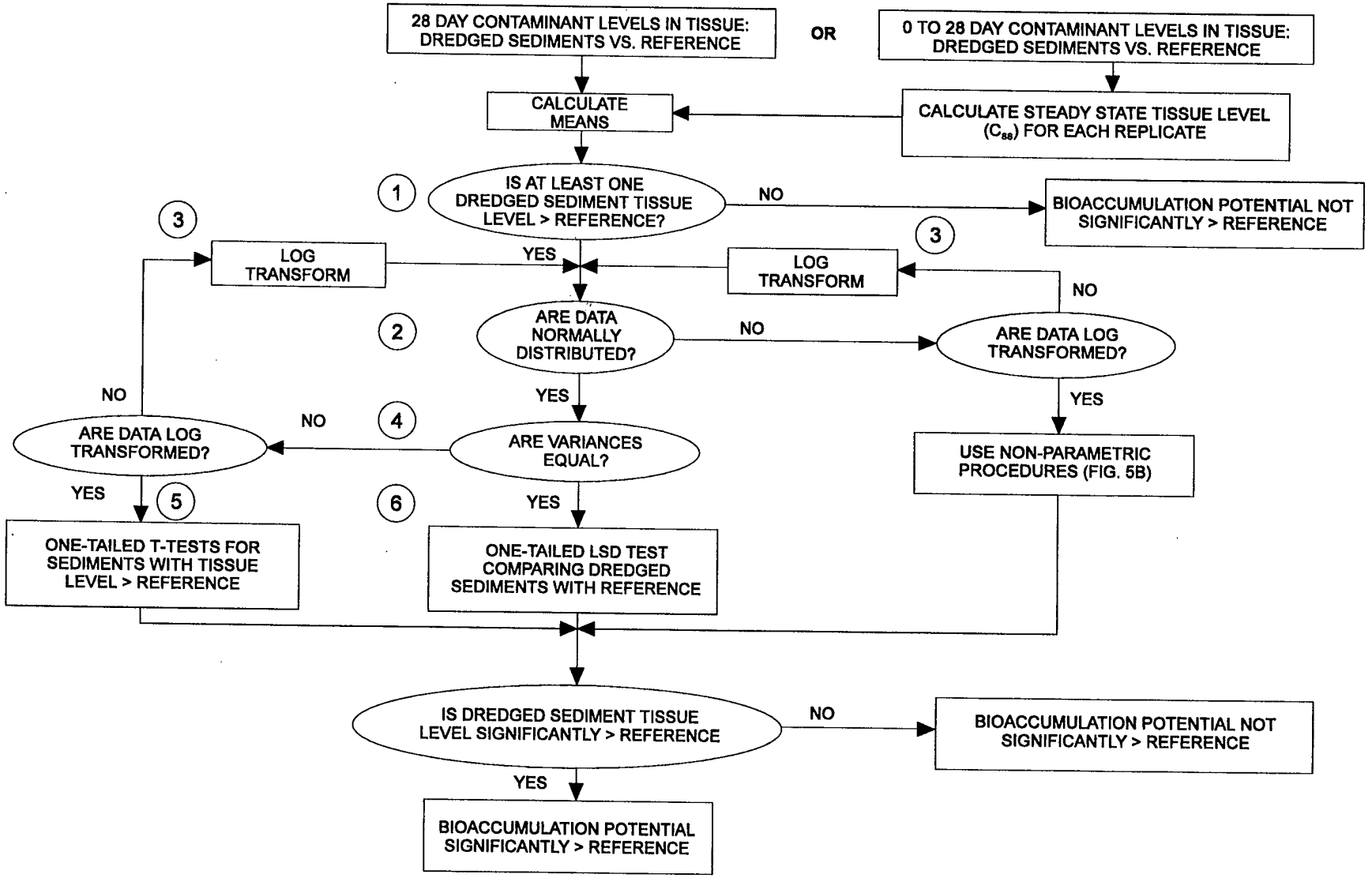


Figure D-5A. Bioaccumulation Test Decision Tree (Parametric Tests).

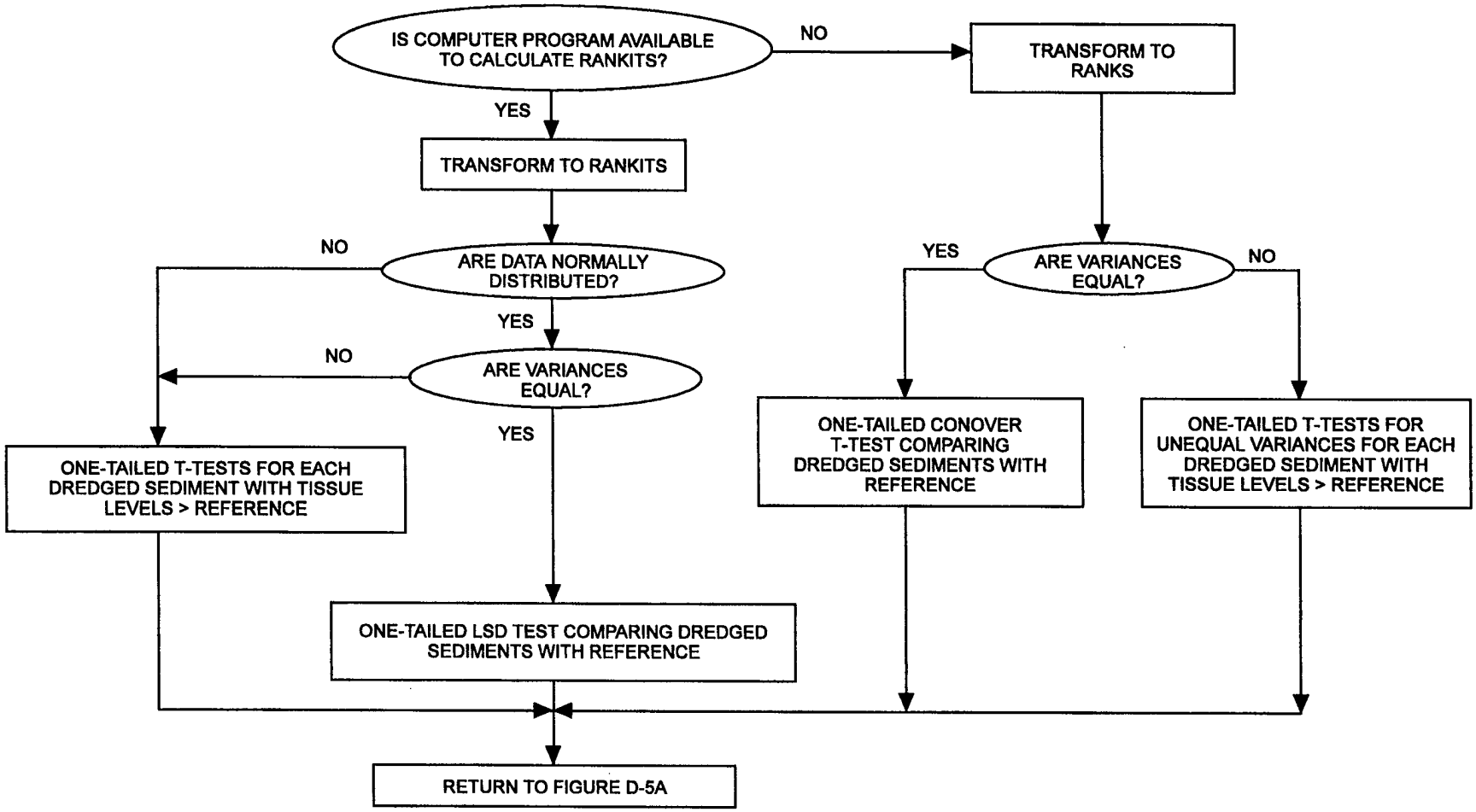


Figure D-5B. Bioaccumulation Test Decision Tree (Nonparametric Tests).

The data in Table D-9 were analyzed using SAS program BIOACC (Section D4.3), and the results are reported in Tables D-10 and D-11. The probability value for Shapiro-Wilk's Test (*2*) was >0.01 (α level in Table D-2 for N=20, balanced data), indicating no significant departure from normality. If the raw data had failed the normality test, then a log transformation (*3*) would be applied and the Shapiro-Wilk's Test rerun (*2*). If the log-transformed data still departed significantly from normality, then nonparametric hypothesis testing procedures would be performed (Figure D-5B); these procedures are described in Section D2.2.1.

The P-value for Levene's Test (*4*) was >0.10 (α level in Table D-2, n=5, balanced data), indicating that assumption of equality of variances need not be rejected for the raw data. If the variances had been significantly unequal, a log transformation would have been applied (*3*) and the tests of assumptions (*2,4*) rerun. Data that passed the normality test but failed the test for equality of variances would be analyzed using a t-test for each dredged sediment-reference sediment comparison (*5*).

Because the example data passed both tests of assumptions, the LSD (*6*) was conducted on the untransformed data to compare bioaccumulation from each dredged sediment with bioaccumulation from the reference sediment. LSD results indicated that mean tissue levels for organisms exposed to dredged sediments 1 and 2 (but not 3) were significantly greater than mean tissue levels for organisms exposed to the reference sediment (Table D-10).

For the sake of illustration, Table D-10 also includes results for log-transformed example data and for t-tests. Table D-11 gives nonparametric test results for the example data. Note that the different statistical tests give conflicting hypothesis test conclusions for the sediment 3-reference sediment comparison, because the P-values of the tests are close to α. This situation will often arise in the analysis of actual bioaccumulation data. Once again, *it is not acceptable to conduct several different statistical tests in order to choose the results one prefers.* For dredged sediment disposal evaluations, the decision trees in this Appendix should be followed to determine the appropriate statistical procedures in any given situation. In the case of the example data, the tests of assumptions indicate that the appropriate hypothesis testing procedure is the LSD test using untransformed data, and the results of this test should be accepted. However, in making decisions concerning disposal, it is entirely appropriate to consider that the significance of the sediment 3-reference sediment comparison is marginal. The power of the LSD test (calculated below) should also be taken into consideration.

Power calculations for the example data are performed on the untransformed data. Using Eq. 17, the minimum significant difference for the parametric LSD test was:

$$d_{min} = 1.746[2(0.003763)/5]^{½} = 0.0677 \text{ µg/g}.$$

SAS conveniently provides this value as the "Least Significant Difference" in the GLM or ANOVA procedures when the LSD test is requested (and sample sizes are equal).

Table D-10. Tests of Assumptions and Parametric Hypothesis Tests on Untransformed and Log_{10}-Transformed Bioaccumulation Example Data.

Null Hypothesis: Mean Dredged Sediment Bioaccumulation Equals Mean Reference Sediment Bioaccumulation[a]				
Test	**Test Statistic**	**Probability *P***	**α**	**Conclusion**
Normality Assumption:				
Shapiro-Wilk's Test				
Untransformed data	*W*=0.958	0.511	0.01	do not reject
Log-transformed data	*W*=0.980	0.921	0.01	do not reject
Equality of Variances Assumption:				
Levene's Test				
Untransformed data	*F*=2.15	0.134	0.10	do not reject
Log-transformed data	*F*=2.19	0.129	0.10	do not reject
Null Hypotheses:				
Sediment 1 = Reference				
LSD Test				
Untransformed data	*t*=3.76	0.0028	0.05	reject
Log-transformed data	*t*=4.45	0.0011	0.05	reject
t-Test (unequal variances)				
Untransformed data	*t*=5.30	0.0020	0.05	reject
Log-transformed data	*t*=7.04	<0.0001	0.05	reject
Sediment 2 = Reference				
LSD Test				
Untransformed data	*t*=3.20	0.0063	0.05	reject
Log-transformed data	*t*=3.84	0.0025	0.05	reject
t-Test (unequal variances)				
Untransformed data	*t*=3.33	0.0129	0.05	reject
Log-transformed data	*t*=4.34	0.0020	0.05	reject
Sediment 3 = Reference				
LSD Test				
Untransformed data	*t*=1.65	0.0688	0.05	do not reject
Log-transformed data	*t*=2.20	0.0295	0.05	reject
t-Test (unequal variances)				
Untransformed data	*t*=2.03	0.0523	0.05	do not reject
Log-transformed data	*t*=1.98	0.0495	0.05	reject

[a] Based on tests of assumptions, appropriate statistical tests of null hypotheses are underlined. Other test results are included for illustration only.

Table D-11. Tests of Assumptions and Nonparametric Hypothesis Tests on Bioaccumulation Example Data Converted to Rankits and Ranks.

Null Hypothesis: Median Dredged Sediment Bioaccumulation Equals Median Reference Sediment Bioaccumulation				
Test	**Test Statistic**	**Probability *P***	**α**	**Conclusion**
Normality Assumption:				
Shapiro-Wilk's Test (rankits)	W=0.972	0.791	0.01	do not reject
Equality of Variances Assumption:				
Levene's Test (rankits)	F=0.61	0.621	0.10	do not reject
(ranks)	F=1.57	0.236	0.10	do not reject
Null Hypotheses:				
Sediment 1 = Reference				
LSD Test (rankits)	t=3.87	0.0024	0.05	reject
t-Test (rankits, unequal variances)	t=4.69	0.0011	0.05	reject
Conover T-Test	t=4.14	0.0016	0.05	reject
t-Test (ranks, unequal variances)	t=6.18	0.0003	0.05	reject
Sediment 2 = Reference				
LSD Test (rankits)	t=3.32	0.0053	0.05	reject
t-Test (rankits, unequal variances)	t=3.76	0.0040	0.05	reject
Conover T-Test	t=3.54	0.0038	0.05	reject
t-Test (ranks, unequal variances)	t=3.95	0.0046	0.05	reject
Sediment 3 = Reference				
LSD Test (rankits)	t=1.66	0.0677	0.05	do not reject
t-Test (rankits, unequal variances)	t=1.69	0.0706	0.05	do not reject
Conover T-Test	t=1.86	0.0497	0.05	reject
t-Test (ranks, unequal variances)	t=1.85	0.1215	0.05	do not reject

Using Eq. 18, the power of the LSD test for detecting a 100% increase in dredged sediment bioaccumulation over the mean reference bioaccumulation (i.e., d=0.066 µg/g) can be determined by:

$$t_{1-\beta} = (0.066)\ [5/2(0.003763)]^{½} - 1.746 = -0.045$$

and 1-β for t=-0.045 with 16 df is 0.48. Power values for 10, 25, 50, 100, 200 and 300% increases over mean reference bioaccumulation are given in the output for SAS program BIOACC (Section D4.3.2).

The sample size (n) required to provide a 0.95 probability (1-β=0.95) of detecting a 25% increase (0.0165 µg/g) over the mean reference bioaccumulation, calculated using the z-approximation (Eq. 9) with MSE replacing s^2, is:

$$n = 2(1.645 + 1.645)^2[0.003763/(0.0165)^2] + 0.25(1.645)^2 = 300\ !$$

Using the same equation, to detect a 100% increase (0.066 µg/g) over the mean reference bioaccumulation with a power of 0.95, n = 20. Assuming we are limited to 5 replicates, there is a 0.95 probability of detecting a

difference (d) of 0.135 µg/g, which is a 205% increase over the mean reference bioaccumulation. Other values of d when power = 0.5, 0.6, 0.7, 0.8, 0.9, and 0.99 are given in the output for SAS program BIOACC (Section D4.3.2).

D3.1.1.1 Less Than Detection Limit Data

A number of methods can be used to permit statistical comparisons of censored data. A simulation study was conducted to identify which of 10 censored data methods work best to maintain power and minimize α in LSD comparisons when n is small, for various situations depending on type of frequency distribution, equality or inequality of variances, coefficient of variation (CV), and amount of censoring (Clarke, 1995a). The 10 censored data methods include three simple substitution methods, two uniform distribution substitution methods, three maximum likelihood methods, and two regression methods. General results from all simulations combined indicate that the simple substitution methods perform as well as or better than the more complicated censored data techniques in most situations (Clarke, 1994). In particular, substitution of the detection limit when up to 40 percent of the data are nondetects, or one-half the detection limit when more than 40 percent of the data are nondetects, are methods that work reasonably well for small sample sizes in most cases.[2] These methods are not limited to untransformed data, but may also be used when data will subsequently be log-transformed or converted to rankits.

Nevertheless, the simulations have shown that substitution of the detection limit or half the detection limit are not the most advantageous censored data methods for all combinations of frequency distribution and variance characteristics. Detailed guidelines for statistical treatment of less than detection limit data developed from the simulation study are described in Clarke (1995b); investigators wishing to maximize the effectiveness of statistical comparisons that include nondetects are encouraged to read this publication carefully. The guidelines are summarized below; the recommendations table from Clarke (1995b) is condensed as Table D-12 and includes the following methods:

- **DL**. Substitution of the detection limit for all nondetects.
- **DL/2**. Substitution of one-half the detection limit for all nondetects.
- **ZERO**. Substitution of zero for all nondetects.

When data are subsequently transformed to rankits, the above three methods produce the exact same results (assuming all uncensored observations in the sample are > DL), and are called **CONST** for substitution of any constant between 0 and DL.

[2]Power will generally decline as censoring increases; when the data are more than 60 to 80 percent nondetects, it is unlikely that any method will perform acceptably.

Table D-12. Recommended Censored Data Methods for Small Samples to be Used in Statistical Comparisons.

Amount of Censoring	Variances	Coefficient of Variation	Data Transformation (Distribution)		
			Log (Lognormal)	None (Normal)	Rankit (Nonnormal)
≤20 %	Equal	≤0.25	DL[a]	DL	CONST, UNIF
		0.26 - 1	DL/2, DL	DL/2, ZERO	CONST, UNIF
		0.51 - 1	*DL/2*, DL	ZERO, DL/2	CONST, UNIF
		>1	DL/2, DL	--[b]	CONST, UNIF
	Increase as Means Increase		DL, DL/2	LR, DL/2	CONST, UNIF
	Mixed		*DL*[c]	DL, DL/2	CONST, UNIF[c]
21 - 40 %	Equal	≤0.25	DL	DL	CONST, UNIF
		0.26 - 1	DL/2	DL/2, ZERO	CONST, UNIF
		>1	DL/2, DL	--[b]	CONST, UNIF
	Increase as Means Increase		DL/2, DL	DL, DL/2	CONST, UNIF
	Mixed		DL	*ZERO, DL/2*[c]	CONST, UNIF
41 - 60 %	Equal	≤0.25	DL/2, DL	DL/2, ZERO	CONST
		>0.25	DL/2	DL/2, ZERO	CONST
	Increase as Means Increase		DL/2	DL/2, ZERO	CONST
	Mixed		DL/2	--[d]	CONST
61 - 80 %	Equal	≤0.25	*DL/2*, DL	DL/2	CONST
		0.26 - 1	DL/2	DL/2, ZERO	CONST
		>1	*DL/2*[c]	--[b]	--[d]
	Increase as Means Increase		DL/2	DL/2, ZERO	CONST
	Mixed		*DL/2*[c]	--[d]	*CONST*[c]

[a] Non-italicized methods have been $\alpha < 0.06$; italicized methods have been α between 0.06 and 0.10

[b] When coefficient of variation > 1, normal distribution is unlikely for chemical conentration data due to increasing proportion of negative values

[c] All methods with accetable power have $\alpha \geq 0.06$

[d] All methods have unacceptably low power and/or high α

- **UNIF.** Nondetects are replaced by ordered observations x_i (i = 1, 2...nc, where nc is the number of censored observations in the sample) between 0 and DL, where

$$x_i = \mathbf{DL}(i - 1)/(nc - 1)$$

and x_i = DL/2 when nc = 1.

- **LR.** Substitution of estimated values from a lognormal distribution using linear regression of logarithms of above-DL concentrations vs their rankits. The regression equation is used to extrapolate values for which antilogs are taken to replace the nondetects. This method (called Helsel's Robust Method) is available in a software package called UNCENSOR[3] (Newman and Dixon, 1990).

SAS program statements for DL, DL/2, ZERO, UNIF, and LR are given in Section D4.5.

Deletion of nondetects is not recommended as it results in excessive loss of information and power as amount of censoring increases.

The following steps should be used to select the best censored data method in a given situation. For each contaminant reporting nondetects:

- Determine proportion of data that are censored (all samples combined).

- Determine characteristics of the variances and statistical data distribution for the contaminant of interest. This can be done if the data are not severely censored by applying two or more censored data methods to obtain a range of possible variances and CVs. Alternatively, one might use uncensored sample data for the same or similar contaminants, or historical data for the same contaminant from the same area.

    - Determine whether variances are equal or unequal among samples (Section D2.1.1.1). If unequal, do the variances increase as means increase, or are the variances seemingly random (mixed)?

    - Calculate CV of combined samples, where CV = $s / \bar{x}$

    - Determine whether combined sample residuals are distributed normally, lognormally, or nonnormally (Section D2.1.1.1). If CV ≥ 1, data are unlikely to be distributed normally as such a population would include a fair proportion of negative concentrations; therefore, assume lognormal or nonnormal distribution

[3]A public domain program that can be obtained from Dr. Michael C. Newman, University of Georgia Savannah River Ecology Laboratory, P.O. Drawer E, Aiken, SC 29801.

- Refer to Table D-12 to determine most appropriate method given approximate amount of data censoring, properties of variances and type of statistical distribution

- If it is crucial to maintain α at approximately 0.05 or less, choose non-italicized methods where available in Table D-12

- Apply selected method to censored data, then continue with tests of assumptions and statistical comparison procedures as outlined in Section D3.1.1. Avoid a data transformation for which no method is given in Table D-12 due to low power or excessively high α

- Do not attempt statistical comparisons of severely censored samples in situations where no censored data methods are considered appropriate. In such cases, the probability of an erroneous outcome is high.

It is quite possible that an evaluation including a number of sediments and contaminants would produce comparisons involving several different combinations of censoring proportions, variance characteristics and data frequency distributions. Following the guidelines herein would result in the application of more than one censored data method to the project data. This is entirely acceptable when the censored data methods are selected for the purpose of maximizing power and minimizing type I error. *What is not acceptable is to try several censored data methods for the purpose of finding one that will produce a desired statistical comparison outcome.*

The simulation study did not address the performance of censored data methods in the common situation of multiple detection limits within a set of replicate observations. Until guidelines are developed for analysis of multiple detection limits, the same procedures should be followed as for single detection limits. SAS programs for the censored data methods can be applied without modification to multiple detection limit censored samples.

D3.1.2 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 sediment is significantly less than a specified action level or standard. If the mean tissue concentration of one or more contaminants of concern is greater than or equal to the applicable action level, then no statistical testing is required. The conclusion would be that the dredged sediment does not meet the guidelines associated with the action level (Section 6.3). If the mean tissue concentrations of a contaminant of concern are less than the applicable action level, then a confidence-interval approach is used to determine if these means are *significantly* less than the action level. One-sided tests are appropriate since there is concern only if bioaccumulation from the dredged sediment is not significantly less than the action level. There are two different approaches to conducting these tests, and both are acceptable.

The first approach is to calculate a value of t, much as in a t-test (this approach is often called a one-sample t-test):

$$t = \frac{\bar{x} - \textit{action level}}{\sqrt{s^2/n}} \quad , \qquad \textbf{(Eq. 20)}$$

where $\bar{x}$, s^2 and n refer to mean, variance, and number of replicates for contaminant bioaccumulation from the dredged sediment.

If tests of equality of variances in the comparison of dredged sediments with the reference indicate that variances are equal for all sediments, then *MSE* from the ANOVA is used as s^2, and calculated t is compared to $t_{0.95}$, with $N - k$ degrees of freedom. If the variances are not equal, then s^2 for the individual sediment is used, and calculated t compared with $t_{0.95}$, with $n - 1$ degrees of freedom. If the data were log-transformed to normalize the distributions or equalize variances, then all calculations should be carried out on log-transformed values.

Another approach is to calculate the upper one-sided 95% confidence limit (*UCL*), and compare it to the action level:

$$UCL = \bar{x} + (t_{0.95,v})(\sqrt{s^2/n}) \quad . \qquad \textbf{(Eq. 21)}$$

As in the first approach, the *MSE* is used in place of s^2 if variances are not significantly different, and the degrees of freedom (v) are $N - k$. If variances are significantly different, s^2 for the individual sediment is used, and v for each sediment i is $n_i - 1$. There is a 0.95 probability that the true population mean tissue level is below the *UCL*. If the *UCL* is below the action level, there is a ≥0.95 probability that the population mean tissue level for the dredged sediment is below the action level, and we conclude that the action level is not exceeded. If the *UCL* is above the action level, we cannot be sure that the mean population tissue level does not exceed the action level.

Either of the above procedures may be used with data that have failed the normality test, but the results should be considered approximate.

The choice of which approach to use depends on the computer software and the presentation method to be used. In SAS, it is more convenient to calculate the *UCL* and compare with the action level, as in program BIOACC (Section D4.3). In SYSTAT, it is simpler to conduct a one-sample t-test. Both approaches can easily be performed by hand. If the data are presented graphically, as in Figure D-6, the confidence-level approach is used. If the investigator wants to provide the exact probability that the mean tissue level is less than the action level, then the one-sample t-test is used.

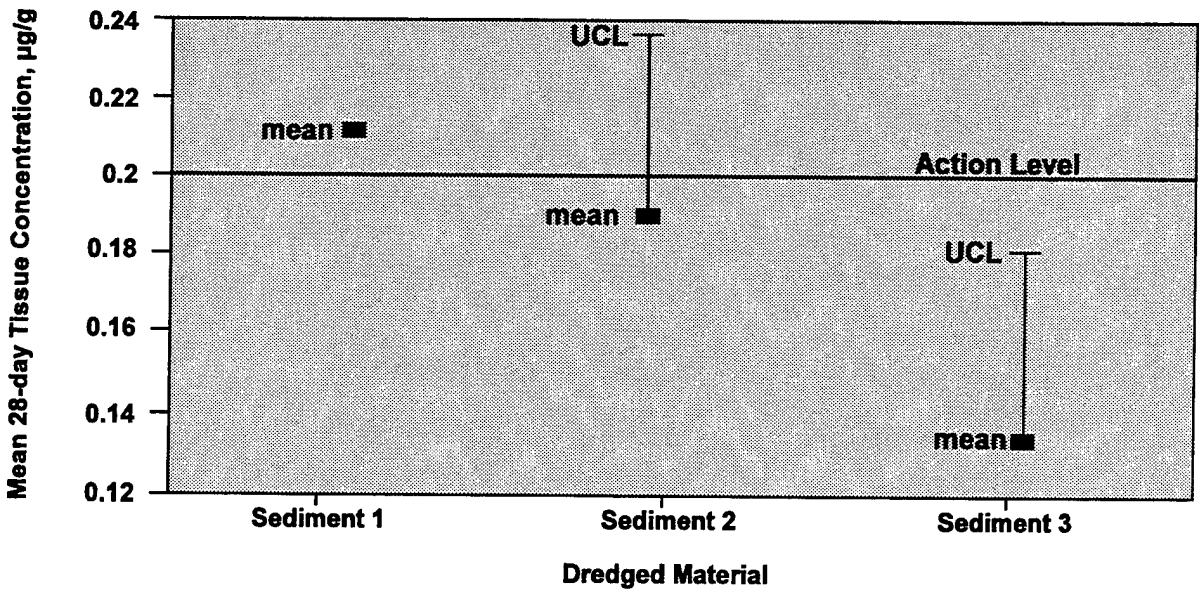


Figure D-6 Comparison of Mean Dredged Sediment Contaminant Tissue Levels (mean) and 95% Upper Confidence Level (*UCL*) with Hypothetical Action Level.

Figure D-6 presents a comparison of mean bioaccumulation from the three dredged sediments (see Table D-9) with a hypothetical action level of 0.2 µg/g. There is no need to calculate the *UCL* for sediment 1 as the mean exceeds the action level. Because variances were not significantly different for the untransformed data (Table D-10), we use MSE=0.003763 and $t_{0.95,16}$=1.746 in Eq. 21 to obtain:

$$UCL = 0.190 + 1.746(0.003763/5)^{½} = 0.238$$

for sediment 2, and UCL = 0.178 for sediment 3. SAS program BIOACC (Section D4.3) calculates *UCL* for both equal and unequal variances.

If the *UCL* lies below the action level, there is a >0.95 probability that the true population mean tissue level for that sediment is less than the action level. Thus, we would conclude that mean bioaccumulation for dredged sediment 3 is less than the action level. Because the *UCL* for sediment 2 exceeds the action level even though the sample mean does not, we cannot be sure that the true population mean tissue level for this sediment is less than the action level.

Formulae for calculating statistical power for comparisons to a fixed standard such as an action level are very similar to Eqs. 8 and 9:

$$n = (t_{1-\alpha,\nu} + t_{1-\beta,\nu})^2 (s^2/d^2) , \qquad \textbf{(Eq. 22)}$$

where s^2 and ν (degrees of freedom) are *MSE* and $N - k$ if variances are equal (or expected to be equal, if the calculation is made prior to testing), and s^2 for the individual sediment and $n_i - 1$ if variances are unequal. It is usually easier to use the z-approximation (from Alldredge, 1987) to avoid solving for n iteratively:

$$n = (z_{1-\alpha} + z_{1-\beta})^2 (s^2/d^2) + 0.5(z_{1-\alpha}^2) \quad . \qquad \textbf{(Eq. 23)}$$

The formulae indicate that the sample size required to detect any given difference d will be approximately one-half that required for a comparison of two treatments. The sample size required is lower because the comparison is made to a fixed value, rather than to a reference which can also vary. Thus, there is no sampling uncertainty or error for the fixed standard and we know the true value of one of the two things being compared.

Using the z-approximation and s^2=*MSE*, the sample size required to provide a 0.95 probability (1-β=0.95) of detecting a tissue level 25% (0.05 µg/g) below the action level is:

$$n = (1.645 + 1.645)^2(0.003763)/0.0025 + 0.5(1.645)^2 = 18.$$

The minimum significant difference is:

$$d_{min} = t_{0.95,16}(MSE/n)^{½} = 1.746(0.003763/5)^{½} = 0.048 \text{ µg/g}.$$

The power of a comparison can be determined by:

$$t_{1-\beta} = \frac{d\sqrt{n}}{s} - t_{1-\alpha,\nu} \quad , \qquad \textbf{(Eq. 24)}$$

When variances are not significantly different, s is replaced by $(MSE)^{½}$ and $\nu = N - k$ df. Using *MSE*=0.003763 as above, the power to detect a 10% decrease in mean bioaccumulation below the action level is 0.16, and power to detect a 50% decrease is 0.96. Power for 10, 20, 30, 40 and 50% decreases are given in the output for SAS program BIOACC (Section D4.3.2).

D3.1.2.1 Less Than Detection Limit Data

Recommendations for censored data methods in Table D-12 were developed to facilitate comparisons of two or more samples. When a sample of contaminant bioaccumulation concentrations must be compared with an action level or standard, accurate estimation of the sample mean and standard deviation is important. In general, this may require different censored data methods than does the comparison of samples in the previous section. Most recommendations for censored data methods in estimation

problems have been based on relatively large sample sizes (n=10 or more). Gleit (1985) identified certain methods that perform better than others for estimating the mean and variance of normal populations based on samples of n=5. The best methods, depending on mean, CV, and amount of censoring, included DL, DL/2, ZERO, and an iterative method using expected values of order statistics. The latter method (which Gleit recommended), along with several others including LR and some MLE techniques, are available in UNCENSOR (Newman and Dixon, 1990).

Recommendations for censored data methods for estimating mean and standard deviation when n is small are provided in the Applications Guide as a supplement to this Appendix (Clarke and Brandon, in press). If zero is substituted for all nondetects and the sample mean is greater than or equal to the applicable action level, then clearly no statistical testing is required as the mean contaminant concentration cannot be less than the action level.

D3.2 Tier IV Time-Sequenced Laboratory Bioaccumulation Study

The time-sequenced laboratory bioaccumulation test in Tier IV is designed to detect differences, if any, between steady-state bioaccumulation in organisms exposed to the dredged sediments 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 contaminant between sediment and organism is zero.

A simple kinetic model (McFarland and Clarke, 1987; Clarke and McFarland, 1991) 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:

$$C_t = \frac{k_1 C_w}{k_2} (1 - e^{-k_2 t}) \quad , \qquad \textbf{(Eq. 25)}$$

where C_t = concentration of a compound in tissues of an organism at time t,
k_1 = uptake rate constant,
C_w = exposure (water) concentration of the compound,
k_2 = elimination rate constant, and
t = time in days.

Using this model, contaminant uptake occurs rapidly at first, and then the rate of uptake gradually diminishes as uptake begins to level off and approach an asymptote (steady state).

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:

$$C_t = \frac{k_1 C_w}{k_2} = C_{ss} \quad , \qquad \textbf{(Eq. 26)}$$

where C_{ss} is an estimate of the whole-body concentration of the compound at steady state.

Steady-state concentration estimates from organisms exposed to dredged sediments are compared to applicable action levels and to steady-state estimates from organisms exposed to the reference sediment. The data analysis involves several steps:

1. Calculate a separate nonlinear regression for each replicate using Eq. 25.

2. Use the regression coefficients (k_1 and k_2) to calculate the steady-state concentrations (C_{ss}) from Eq. 26, or set up the regression analysis to estimate/output C_{ss} directly (see below).

3. Use the estimates of C_{ss} as data in a statistical test comparing each dredged sediment to the reference (as in Section D3.1.1). Conclusions possible from these comparisons and evaluative factors that should be assessed are detailed in Section 6.3.

4. Use confidence intervals or one-sample *t*-tests to compare the steady-state estimates with applicable action levels (as in Section D3.1.2).

If nondetects occur in the early days of uptake, values may be assigned to them using a censored data method such as DL/2. If nondetects occur in the later portion of uptake, or if all of the bioaccumulation data for a replicate are near the detection limit, then the data probably do not fit the simple kinetic model and that replicate should be dropped from the analysis.

D3.2.1 Calculating Steady-State Concentrations

Table D-13 presents example data resulting from a hypothetical 28-day time-sequenced laboratory bioaccumulation test using three dredged sediments and a reference sediment. There are five replicates of each treatment, and tissue samples were analyzed on days 2, 4, 7, 10, 18, and 28 of the test. More sampling days are scheduled in the early part of the test to enable more accurate characterization of the early, rapidly changing portion of the uptake curve.

Table D-13. Results from a Hypothetical Time-Sequenced Bioaccumulation Test, Showing Contaminant Concentrations (µg/g) in Tissues of Animals Exposed to Different Treatments.

Replicate	Day	Treatment			
		Reference	Sediment 1	Sediment 2	Sediment 3
1	2	0.054	0.159	0.869	0.745
	4	0.441	0.516	0.838	1.316
	7	0.687	0.881	1.246	1.583
	10	0.037	0.278	1.767	1.578
	18	0.856	0.904	1.631	2.822
	28	0.514	0.172	1.178	1.295
2	2	0.163	0.292	0.726	1.703
	4	0.797	0.158	0.633	0.930
	7	0.177	0.317	0.816	2.715
	10	0.549	0.485	1.272	2.268
	18	0.598	1.300	1.877	2.607
	28	0.839	1.049	1.721	2.964
3	2	0.391	0.428	0.394	2.045
	4	0.203	0.743	0.452	2.141
	7	0.862	0.270	0.897	1.016
	10	0.884	0.051	1.003	1.756
	18	0.016	0.671	1.487	3.414
	28	0.793	0.476	1.366	2.109
4	2	0.234	0.558	1.232	1.855
	4	0.564	0.324	0.728	1.150
	7	0.413	0.562	1.639	2.221
	10	0.787	0.909	1.158	2.899
	18	0.806	0.934	1.216	1.319
	28	0.899	0.712	1.513	2.820
5	2	0.034	0.256	0.977	1.135
	4	0.018	0.126	1.314	1.621
	7	0.029	0.603	0.688	2.134
	10	0.294	0.718	1.415	0.890
	18	0.119	1.173	1.280	1.866
	28	0.226	1.245	1.843	3.325
Mean Sediment Concentration		**0.45**	**4.0**	**33.0**	**44.0**

These data can be used with iterative nonlinear regression methods such as those in the SAS NLIN or SYSTAT NONLIN procedures to solve for the parameters (k_1 and k_2) in the model above. Then C_{ss}, the steady-state concentration, is simply k_1C_w/k_2. In this iterative calculation method, the contaminant concentration in the sediment (C_s), or even a constant such as 1, may be used instead of C_w. This is because the values of the rate constants and the exposure concentration are not of interest in this application, only their ratio (i.e., C_{ss}). Thus, the equation could be written as:

$$C_t = C_{ss} (1 - e^{-k_2 t}) \quad , \qquad \textbf{(Eq. 27)}$$

and C_{ss} estimated directly by the regression software. The estimate of C_{ss} should be the same regardless of which approach is used. SAS program BIOACCSS (Section D4.4) performs the steady-state calculations using C_s, and outputs the regression parameters and C_{ss} for each replicate to a new data set. These are displayed in Table D-14.

Nonlinear regressions for the example data were calculated using the SAS NLIN procedure with the DUD method. This method does not require derivatives. Other methods may be used but the derivatives for the parameters (k_1 and k_2, or C_{ss} and k_2 if C_{ss} is estimated directly) must be specified. The Marquardt method and the Gauss-Newton method produced results similar to DUD for the example data.

Iterative curve-fitting techniques will provide better fits to some data than to others, and the asymptotic relationship will not always be the best fit to the data. Thus, investigators should be aware of the following problems:

1. Failure to converge on a solution within the allowed number of iterations. Always have the regression software print out the results, even though the regressions are only used to create a new data set of C_{ss} values. SAS will output the parameter estimates from the final iteration, regardless of whether convergence occurred. If the last few iterations approach convergence (i.e., there is little change in parameter estimates and residual error mean square), then the parameter estimates from the last iteration may be used. If convergence was not approached, then the program should be run again for that replicate, using the parameter values from the last iteration as starting values.

2. No relationship between concentration and time. This can occur in sediments with low or non-detectable contaminant levels. The model-derived estimate of C_{ss} will usually converge on the mean tissue concentration over all days.

3. A non-asymptotic relationship. If the relationship between tissue levels and time is linear, rather than asymptotic, the estimated asymptote (C_{ss}) will approach infinity. A linear relationship will occur if the experiment was not conducted for a long enough time for the tissue levels to approach the asymptote, or because of anomalously high tissue levels later in the experiment. Always plot the data prior to calculating the regressions to make sure the relationships are asymptotic. SAS program

Table D-14. Regression Parameters Estimated from Example Time-Sequenced Bioaccumulation Data.

Treatment	C_s µg/g	Replicate	k_1	k_2	C_{ss}
Reference	0.45	1	0.237	0.176	0.608
		2	0.306	0.201	0.687
		3	0.540	0.407	0.597
		4	0.318	0.162	0.883
		5	0.045	0.087	0.234
					Mean = 0.602
					SE = 0.105
Sediment 1	4.0	1	0.059	0.427	0.554
		2	0.019	0.047	1.644
		3	0.243	2.206	0.441
		4	0.051	0.243	0.833
		5	0.024	0.060	1.600
					Mean = 1.014
					SE = 0.256
Sediment 2	33.0	1	0.014	0.319	1.488
		2	0.007	0.113	1.907
		3	0.006	0.120	1.511
		4	0.034	0.878	1.290
		5	0.023	0.568	1.350
					Mean = 1.509
					SE = 0.108
Sediment 3	44.0	1	0.011	0.250	1.964
		2	0.015	0.236	2.776
		3	0.094	1.977	2.087
		4	0.024	0.458	2.259
		5	0.008	0.139	2.648
					Mean = 2.347
					SE = 0.158

BIOACCSS (Section D4.4) provides separate plots for each treatment, with the replicates identified. Anomalies/outliers and non-asymptotic relationships for any replicate can easily be spotted using plots such as these.

If relationships for only one or a few replicates are non-asymptotic, then those replicates can be dropped from the analysis, or the maximum measured tissue concentrations used as an estimate of C_{ss}. If relationships for several replicates (i.e., >5 total, or >1 for any individual sediment) are non-asymptotic, then there is little justification for assuming that a steady state has been approached. The test should be repeated, but over a longer time interval. Measuring concentrations in field-collected organisms is also an alternative, if steady state is not reached in laboratory experiments (see Section D3.3).

4. Estimates of C_{ss} that are negative. This can happen if tissue concentrations decrease over time and k_2 is negative. If there are only one or a few replicates with negative C_{ss} values, then these replicates can be dropped from the analysis. Alternatively, the minimum or mean measured concentration could be used as an estimate of C_{ss}. If there are several (i.e., >5 total, or >1 for any individual sediment), then the test should be repeated. High initial contaminant levels in the test organisms are the most probable cause of negative C_{ss} values. Prior to repeating the test, these initial contaminant levels should be measured, and the source of test organisms should be changed if these levels are greater than bioaccumulation of the contaminant at the end of the previous test.

If difficulties are encountered, approaches such as those discussed by Draper and Smith (1981) and SCI (1989) should be considered. Investigators with limited experience should always consult an applied statistician familiar with nonlinear regression prior to analyzing time-sequenced bioaccumulation data. It is important to remember that these data are usually very expensive to obtain, because of the extensive number of chemical analyses required, and the data should be carefully and correctly analyzed.

In the example data analysis, the DUD method failed to converge within the default number of iterations (50) for sediment 3, replicate 5. However, the procedure was close enough to convergence that the regression coefficients output at the final iteration produced a reasonable estimate of C_{ss}.

The approach recommended in this Appendix for comparison of Tier IV dredged sediment and reference sediment bioaccumulation data differs from that described in the Ocean Disposal Manual (the "Green Book"). The approach of comparing 95% confidence intervals for C_{ss} is not recommended because:

- The 95% confidence intervals apply to the estimate of C_{ss} rather than to the *difference* between estimates
- The 95% confidence intervals are based on regressions through points from all replicates for a treatment, ignoring variation among replicates within a treatment
- Different programs or methods will provide different confidence intervals for the same data
- Measurements of tissue levels taken at different times may not be independent.

If the objective of the Tier IV investigation is only to compare bioaccumulation from reference and dredged sediments over the duration of the experiment, and estimates of C_{ss} are not required, there are other alternatives to analyze the data:

- Repeated measures analysis of variance, testing for linear and quadratic components of the time trend

- Multivariate analysis of variance (MANOVA), with tissue levels for each day considered separate variables (linear and quadratic trends can also be tested in MANOVA).

These alternatives are equivalent with respect to testing for linear and quadratic trends over time, and some repeated measures programs (e.g., SYSTAT MGLH) will provide MANOVA results as well. These alternatives should only be used by experienced investigators who are familiar with them. Both alternatives would be most useful in testing for an overall quadratic trend, as the absence of such a trend over time would indicate that tissue levels did not approach an asymptote within the duration of the experiment.

D3.2.2 Comparison with Reference Sediments and Action Levels

The difficult part of analyzing time-sequenced bioaccumulation tests is obtaining sound estimates of C_{ss}. Once these estimates are obtained, they are analyzed using the same procedures as for single time-point bioaccumulation tests (Section D3.1). Steady-state concentration estimates for the dredged sediments are compared to steady-state concentration estimates for the reference sediment using the appropriate methods from the decision trees in Figures D-5A or 5B.

The values of C_{ss} in Table D-14 were analyzed using the decision tree steps in Figure D-5A. Although SAS Program BIOACCSS (Section D4.4) conducts all parametric and rankit analyses from the decision trees, only the appropriate results are reported in Table D-15. The untransformed C_{ss} values were normally distributed (Shapiro-Wilk's Test, $P>0.01$, the α level from Table D-2 for $N=20$, balanced data). However, neither the untransformed nor log-transformed C_{ss} passed Levene's Test for equality of variances ($P<0.10$, the α level in Table D-2 for $n=5$, balanced data). Therefore, *t*-tests were conducted, comparing each dredged sediment C_{ss} with reference sediment C_{ss}, using the untransformed C_{ss} estimates. Note that *t*-tests for equal variances could be used because the F' tests for each dredged sediment-reference comparison did not reject equal variances, even though the overall test of equality of variances indicated unequal variances within the data set as a whole. Mean estimated concentrations at steady state for dredged sediments 2 and 3 (but not sediment 1) were significantly greater than that of the reference sediment (Table D-15).

Table D-15. Tests of Assumptions and Parametric Hypothesis Tests on Untransformed Steady-State Bioaccumulation Example Data.

Null Hypothesis: Mean Dredged Sediment Steady-State Bioaccumulation Equals Mean Reference Sediment Steady-State Bioaccumulation				
Test	**Test Statistic**	**Probability *P***	**α**	**Conclusion**
Normality Assumption:				
Shapiro-Wilk's Test				
Untransformed data	*W*=0.963	0.613	0.01	do not reject
Log-transformed data	*W*=0.943	0.280	0.01	do not reject
Equality of Variances Assumption:				
Levene's Test	*F*=4.74	0.015	0.10	reject
Untransformed data	*F*=3.68	0.034	0.10	reject
Log-transformed data				
Null Hypotheses:				
Sediment 1 = Reference				
t-Test (equal variances)	*t*=1.49	0.0873	0.05	do not reject
Sediment 2 = Reference				
t-Test (equal variances)	*t*=6.03	0.0002	0.05	reject
Sediment 3 = Reference				
t-Test (equal variances)	*t*=9.21	<0.0001	0.05	reject

Power calculations for an LSD test using untransformed data are performed in SAS program BIOACCSS (Section D4.4). From Eq. 18, a 50% increase over the mean reference C_{ss} (0.602 µg/g) can be detected with a probability of 0.32, and a 100% increase with a probability of 0.78. Likewise, there is a 0.95 probability of detecting a 138% increase in C_{ss} over the mean reference C_{ss}. The least significant difference from the LSD is 0.415 µg/g, which is a 69% increase over the mean reference C_{ss}. Sample size (n) required to provide a 0.95 probability of detecting a 25% increase over the mean reference C_{ss} is 136 (Eq. 9, using MSE in place of s^2).

The C_{ss} values for the dredged sediment can also be compared to an action level, if available, using the one-sample t-test or one-sided upper confidence limits (UCL) as in Section D3.1.2. UCL for both equal variances and unequal variances may be calculated using SAS program BIOACCSS (Section D4.4). Figure D-7 provides the mean C_{ss} and UCL for each example dredged sediment, along with a hypothetical action level of 2 µg/g. The UCL for sediments 1 and 2 were below the action level, indicating that the C_{ss} for these sediments were significantly lower than the action level. The mean C_{ss} for sediment 3 was above the action level, so there was no need to calculate a UCL to conclude that the C_{ss} was not significantly lower than the action level.

Power to detect a true population steady-state concentration 10, 20, 30, 40 and 50% below an action level is calculated in SAS program BIOACCSS (Section D4.4).

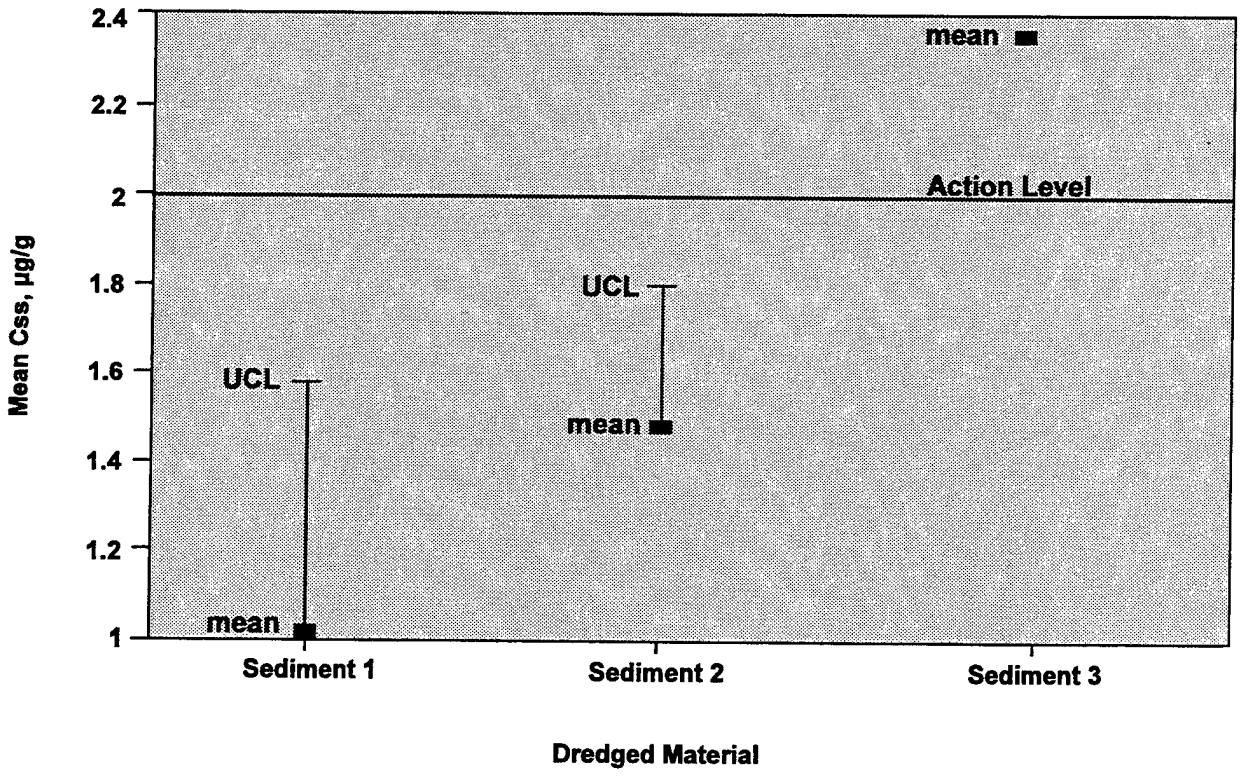


Figure D-7. Comparison of Mean Dredged Sediment Contaminant Steady-State Tissue Levels (C_{ss}) (mean) and 95% Upper Confidence Levels (UCL) with Hypothetical Action Level.

D3.3 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.

Replicate tissue concentrations in organisms collected at the disposal site(s) are compared with replicate tissue concentrations in organisms collected from the reference area using the decision tree steps in Figures D-5A and 5B. If comparisons involve organisms from only one disposal site, then the appropriate statistical comparison procedures, depending on the results of the tests of assumptions, are the two-sample *t*-test for equal or unequal variances, or the *t*-test for unequal variances using rankits or ranks (Section D2.1.1.1).

D4.0 SAS PROGRAMS AND OUTPUT FOR EXAMPLE DATA

This Section provides SAS programs to analyze the example data sets given in Appendix D. Each program includes all analyses from the corresponding decision tree that would be performed using SAS. While it is certainly possible to conduct the statistical analysis of a data set in a stepwise fashion, we find it much more efficient to perform all analyses at once, and then select the appropriate results based on the steps in the decision tree. Power calculations are provided in addition to the decision tree analyses.

SAS statements in the sections that follow are given in uppercase letters (although this is not required for SAS). Comments within the body of the programs are in upper and lowercase letters in the following format: /* Comment line. */. Every SAS statement must end with a semicolon, but several statements may be included on the same line. The programs are designed for the analysis of Appendix D example data, but can be used with other data sets after minor modifications. Investigators wishing to use these programs should have some familiarity with SAS. SAS output follows each program; the output has been edited to remove much of the nonessential information.

We recommend that data analysis reports include at least the following:

- Number of replicates, mean and SE for each treatment
- Treatment of less-than detection limit data, if any
- Results of tests of assumptions
- Data transformation used, if any
- Name of statistical hypothesis testing procedure, its calculated test statistic and associated probability, and conclusion reached regarding the null hypothesis

- Minimum significant difference or some other indication of power for a parametric LSD test or *t*-test.

SAS programs and output are also provided for censored data methods used when bioaccumulation data include nondetects.

D4.1 Program WATTOX.SAS for Water Column Toxicity Test Data Analysis

WATTOX.SAS is a program to compare dilution water survival vs. 100% elutriate survival, using an arcsine-square root transformation on the data. The program performs all statistical analyses in Figure D-1. Included in these analyses are: mean survival for dilution water and elutriates, Shapiro-Wilk's Test for normality, *t*-test for equal or unequal variances, and a *t*-test for unequal variances on data converted to rankits. Refer to the decision tree in Figure D-1 to determine which test results should be used. Minimum significant difference and some other power calculations for the parametric *t*-test are also provided.

D4.1.1 WATTOX.SAS Program Statements

```
LIBNAME Q 'C:\SAS';
OPTIONS LINESIZE=79 PAGESIZE=59 NODATE NONUMBER;

/* Identify the treatment codes. */

PROC FORMAT;
 VALUE TRTFMT
   0='DILUTION WATER '
   1='100% ELUTRIATE '
   2='50% ELUTRIATE  '
   3='25% ELUTRIATE  '
   4='12.5% ELUTRIATE';

/* Input the toxicity test data after the CARDS statement, listing the      */
/* treatment code, replicate, and number of survivors.  A permanent SAS     */
/* data set is created in the directory specified in the LIBNAME statement. */

DATA Q.WATCOL;
 INPUT TRT REP SURV @@;
 CARDS;
0 1 20 0 2 19 0 3 20 0 4 20 0 5 19
1 1 6 1 2 7 1 3 9 1 4 5 1 5 8
2 1 8 2 2 8 2 3 9 2 4 10 2 5 11
3 1 12 3 2 18 3 3 15 3 4 14 3 5 13
4 1 17 4 2 17 4 3 18 4 4 16 4 5 18
;
/* Input no. of organisms (M) per test container at start of test. */
/* Calculate proportion of survivors (SURV/M) and take the SQRT. */
/* Arcsine transform SQRT(SURV/M). */
/* Format, print, sort the data.  Print no. of observations, mean, and */
/* standard error for survival in each treatment. */

DATA A0;
 SET Q.WATCOL;
 M=20;
 ARCSURV=ARSIN(SQRT(SURV/M));
 LABEL TRT='TREATMENT GROUP'
```

```
       REP='REPLICATE'
       M='NO. OF ORGANISMS PER REPLICATE'
       SURV='NUMBER OF SURVIVORS'
       ARCSURV='ARCSINE TRANSFORMATION';
 FORMAT TRT TRTFMT.;
 TITLE 'WATER COLUMN TOXICITY DATA';
PROC PRINT LABEL; VAR TRT REP M SURV ARCSURV;
PROC SORT; BY TRT;
PROC MEANS NOPRINT; BY TRT; VAR SURV;
 OUTPUT OUT=Y N=N SUM=TOTAL MEAN=MEANSURV STDERR=SE;
PROC PRINT LABEL; VAR TRT N MEANSURV SE;
 LABEL MEANSURV='MEAN SURVIVAL';

/* Delete data not needed for the dilution water-100% elutriate comparison. */
/* Print descriptive statistics. */

DATA A;
 SET A0;
 IF TRT>1 THEN DELETE;
 TITLE2 'ARCSINE-SQUARE ROOT TRANSFORMATION';
PROC MEANS NOPRINT; VAR ARCSURV; BY TRT; ID M;
 OUTPUT OUT=X N=N MEAN=MEAN VAR=VARIANCE STD=S STDERR=SE;
PROC PRINT LABEL; VAR TRT N MEAN VARIANCE S SE;

/* Test normality of residuals using Shapiro-Wilk's Test. */

PROC GLM DATA=A NOPRINT;
 CLASS TRT;
 MODEL ARCSURV=TRT;
 OUTPUT OUT=Z R=RESID;
PROC UNIVARIATE NORMAL DATA=Z;
 VAR RESID;
 TITLE3 'SHAPIRO-WILKS TEST';

/* Conduct t-test, which includes F' test for equality of variances. */

PROC TTEST DATA=A;
 CLASS TRT;
 VAR ARCSURV;
 TITLE3 'T-TEST';

/* Convert data to rankits and conduct t-test. */

PROC RANK DATA=A NORMAL=BLOM OUT=A1;
 VAR SURV; RANKS RANKIT;
PROC TTEST DATA=A1;
 CLASS TRT;
 VAR RANKIT;
 TITLE2 'DATA CONVERTED TO RANKITS';

/* Calculate minimum significant difference and power of a t-test to detect */
/* true population differences of 10, 20, 30, 40 and 50% below mean */
/* dilution water survival. */

DATA B0;
 MERGE X Y;
 IF TRT^=0 THEN DELETE;
 MEAN0=MEAN; N0=N; S20=VARIANCE;
 MEANPCT=MEANSURV/M;
DATA B1;
 SET X;
 IF TRT^=1 THEN DELETE;
 N1=N; S21=VARIANCE;
```

```
DATA B2;
 MERGE B0 B1;
 DF=N0+N1-2;
 N=(N0+N1)/2;
 S2POOL=(S20*(N0-1)+S21*(N1-1))/DF;
 TALPHA=TINV(.95,DF);
 DMIN=TALPHA*SQRT(2*S2POOL/N);
 LABEL N='NO. OF REPLICATES'
       MEANPCT='MEAN DILUTION WATER SURVIVAL'
       S2POOL='POOLED VARIANCE'
       DF='DEGREES OF FREEDOM, DF'
       TALPHA='T VALUE FOR (1-ALPHA=0.95,DF)'
       DMIN='MINIMUM SIGNIFICANT DIFFERENCE';
 TITLE2 'POWER OF T-TEST TO DETECT A TRUE POPULATION DIFFERENCE (D)';
 TITLE3 'FROM MEAN DILUTION WATER SURVIVAL USING ARCSINE TRANSFORMATION';
PROC PRINT LABEL NOOBS; VAR M N MEANPCT S2POOL DF TALPHA DMIN;
DATA B3;
 SET B2;
 DO PCTDIFF=10 TO 50 BY 10;
  SEDSURV=MEANPCT-PCTDIFF/100;
  ARCSURV=ARSIN(SQRT(SEDSURV));
  ARCDIFF=MEAN0-ARCSURV;
  TBETA=(SQRT(N)*ARCDIFF)/SQRT(2*S2POOL)-TALPHA;
  POWER=PROBT(TBETA,DF);
  OUTPUT;
  END;
 LABEL PCTDIFF='% REDUCTION IN SURVIVAL FROM DIL. WATER'
       SEDSURV='100% ELUTRIATE SURVIVAL'
       ARCSURV='ARCSINE 100% ELUTRIATE SURVIVAL'
       ARCDIFF='D'
       TBETA='T VALUE FOR (1-BETA,DF)';
PROC PRINT LABEL;
 VAR PCTDIFF SEDSURV ARCSURV ARCDIFF TBETA POWER;
 TITLE;
```

D4.1.2 WATTOX.SAS Program Output

WATER COLUMN TOXICITY DATA

OBS	TREATMENT GROUP	REPLICATE	NO. OF ORGANISMS PER REPLICATE	NUMBER OF SURVIVORS	ARCSINE TRANSFORMATION
1	DILUTION WATER	1	20	20	1.57080
2	DILUTION WATER	2	20	19	1.34528
3	DILUTION WATER	3	20	20	1.57080
4	DILUTION WATER	4	20	20	1.57080
5	DILUTION WATER	5	20	19	1.34528
6	100% ELUTRIATE	1	20	6	0.57964
7	100% ELUTRIATE	2	20	7	0.63305
8	100% ELUTRIATE	3	20	9	0.73531
9	100% ELUTRIATE	4	20	5	0.52360
10	100% ELUTRIATE	5	20	8	0.68472
11	50% ELUTRIATE	1	20	8	0.68472
12	50% ELUTRIATE	2	20	8	0.68472
13	50% ELUTRIATE	3	20	9	0.73531
14	50% ELUTRIATE	4	20	10	0.78540
15	50% ELUTRIATE	5	20	11	0.83548
16	25% ELUTRIATE	1	20	12	0.88608
17	25% ELUTRIATE	2	20	18	1.24905
18	25% ELUTRIATE	3	20	15	1.04720

19	25% ELUTRIATE	4	20	14	0.99116
20	25% ELUTRIATE	5	20	13	0.93774
21	12.5% ELUTRIATE	1	20	17	1.17310
22	12.5% ELUTRIATE	2	20	17	1.17310
23	12.5% ELUTRIATE	3	20	18	1.24905
24	12.5% ELUTRIATE	4	20	16	1.10715
25	12.5% ELUTRIATE	5	20	18	1.24905

OBS	TREATMENT GROUP	N	MEAN SURVIVAL	SE
1	DILUTION WATER	5	19.6	0.24495
2	100% ELUTRIATE	5	7.0	0.70711
3	50% ELUTRIATE	5	9.2	0.58310
4	25% ELUTRIATE	5	14.4	1.02956
5	12.5% ELUTRIATE	5	17.2	0.37417

WATER COLUMN TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION

OBS	TREATMENT GROUP	N	MEAN	VARIANCE	S	SE
1	DILUTION WATER	5	1.48059	0.015257	0.12352	0.055239
2	100% ELUTRIATE	5	0.63126	0.006986	0.08358	0.037379

WATER COLUMN TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
SHAPIRO-WILKS TEST

UNIVARIATE PROCEDURE

Variable=RESID

N	10		
W:Normal	0.846238	Prob<W	0.0507

WATER COLUMN TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
T-TEST

TTEST PROCEDURE

Variable: ARCSURV ARCSINE TRANSFORMATION

TRT	N	Mean	Std Dev	Std Error
DILUTION WATER	5	1.48059096	0.12351878	0.05523928
100% ELUTRIATE	5	0.63126480	0.08358232	0.03737915

Variances	T	DF	Prob>|T|
Unequal	12.7340	7.0	0.0001
Equal	12.7340	8.0	0.0000

For H0: Variances are equal, F' = 2.18 DF = (4,4) Prob>F' = 0.4679

WATER COLUMN TOXICITY DATA
DATA CONVERTED TO RANKITS

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE SURV

TRT	N	Mean	Std Dev	Std Error
DILUTION WATER	5	0.74011839	0.44830825	0.20048954
100% ELUTRIATE	5	-0.74011839	0.55672332	0.24897424

Variances	T	DF	Prob>|T|
Unequal	4.6306	7.7	0.0019
Equal	4.6306	8.0	0.0017

For HO: Variances are equal, F' = 1.54 DF = (4,4) Prob>F' = 0.6850

WATER COLUMN TOXICITY DATA
POWER OF T-TEST TO DETECT A TRUE POPULATION DIFFERENCE (D)
FROM MEAN DILUTION WATER SURVIVAL USING ARCSINE TRANSFORMATION

NO. OF ORGANISMS PER REPLICATE	N	MEAN DILUTION WATER SURVIVAL	POOLED VARIANCE	DEGREES OF FREEDOM, DF	T VALUE FOR (1-ALPHA=0.95,DF)	MINIMUM SIGNIFICANT DIFFERENCE
20	5	0.98	0.011121	8	1.85955	0.12403

OBS	% REDUCTION IN SURVIVAL FROM DIL. WATER	100% ELUTRIATE SURVIVAL	ARCSINE 100% ELUTRIATE SURVIVAL	D	T VALUE FOR (1-BETA,DF)	POWER
1	10	0.88	1.21705	0.26354	2.09166	0.96508
2	20	0.78	1.08259	0.39800	4.10768	0.99830
3	30	0.68	0.96953	0.51106	5.80277	0.99980
4	40	0.58	0.86574	0.61485	7.35888	0.99996
5	50	0.48	0.76539	0.71520	8.86344	0.99999

D4.2 Program BENTOX.SAS for Benthic Toxicity Test Data Analysis

BENTOX.SAS is a program to compare benthic toxicity data from dredged sediments vs. reference sediment, using an arcsine-square root transformation on the data. Included in these analyses are: mean survival from each sediment exposure, Shapiro-Wilk's Test for normality, Levene's test for equality of variances, *t*-tests for equal or unequal variances, LSD test, and tests on rankits (normalized ranks for survival). Refer to the decision tree in Figures D-4A and 4B to determine which test results should be used. The program includes power calculations (on an arcsine-transformed scale) for an LSD test.

D4.2.1 BENTOX.SAS Program Statements

```
LIBNAME Q 'C:\SAS';
OPTIONS LINESIZE=79 PAGESIZE=59 NODATE NONUMBER;

/* Identify the treatment codes. */

PROC FORMAT;
 VALUE TRTFMT
   1='REFERENCE '
   2='SEDIMENT 1'
   3='SEDIMENT 2'
   4='SEDIMENT 3';

/* Input the toxicity test data after the CARDS statement, listing the  */
/* treatment code, replicate, and number of survivors.  A permanent SAS */
/* data set is created in the directory specified in the LIBNAME statement. */

DATA Q.BENTHIC;
 INPUT TRT REP SURV @@;
 CARDS;
1 1 20 1 2 20 1 3 19 1 4 19 1 5 20
2 1 17 2 2 16 2 3 18 2 4 17 2 5 15
3 1 15 3 2 16 3 3 13 3 4 17 3 5 11
4 1 17 4 2 12 4 3 10 4 4 16 4 5 13
;
/* Input no. of organisms (M) per test container at start of test. */
/* Calculate proportion of survivors (SURV/M) and take the SQRT.*/
/* Arcsine transform SQRT(SURV/M). */
/* Format, print, sort the data.  Print no. of observations, mean, and */
/* standard error for survival in each treatment. */

DATA A0;
 SET Q.BENTHIC;
 M=20;
 ARCSURV=ARSIN(SQRT(SURV/M));
 LABEL TRT='TREATMENT GROUP'
       REP='REPLICATE'
       M='NO. OF ORGANISMS PER REPLICATE'
       SURV='NUMBER OF SURVIVORS'
       ARCSURV='ARCSINE TRANSFORMATION';
 FORMAT TRT TRTFMT.;
 TITLE 'BENTHIC TOXICITY DATA';
PROC RANK NORMAL=BLOM OUT=A;
 VAR SURV; RANKS RANKIT;
PROC PRINT LABEL; VAR TRT REP M SURV ARCSURV RANKIT;
 LABEL RANKIT='NORMALIZED RANK FOR SURVIVAL';
PROC SORT; BY TRT;
PROC MEANS NOPRINT; BY TRT; VAR SURV; ID M;
 OUTPUT OUT=Y N=N SUM=TOTAL MEAN=MEANSURV STDERR=SE;
PROC PRINT LABEL; VAR TRT N TOTAL MEANSURV SE;
 LABEL MEANSURV='MEAN SURVIVAL';

/* Print descriptive statistics for the arcsine-transformed survival data. */

PROC MEANS NOPRINT DATA=A; VAR ARCSURV; BY TRT;
 OUTPUT OUT=X N=N MEAN=MEAN VAR=VARIANCE STD=S STDERR=SE;
 TITLE2 'ARCSINE-SQUARE ROOT TRANSFORMATION';
PROC PRINT LABEL; VAR TRT N MEAN VARIANCE S SE;

/* Test normality of residuals using Shapiro-Wilk's Test. */
```

```
PROC GLM DATA=A NOPRINT;
 CLASS TRT;
 MODEL ARCSURV=TRT;
 OUTPUT OUT=Z R=RESID;
PROC UNIVARIATE NORMAL DATA=Z;
 VAR RESID;
 TITLE3 'SHAPIRO-WILKS TEST FOR NORMALITY';

/* Conduct Levene's Test for equality of variances. */

DATA AX;
 MERGE A X; BY TRT;
 ABSDEV=ABS(ARCSURV-MEAN);
 LABEL ABSDEV='ABSOLUTE DEVIATIONS FROM MEAN';
PROC GLM;
 CLASS TRT;
 MODEL ABSDEV=TRT;
 TITLE3 'LEVENE''S TEST FOR EQUALITY OF VARIANCES';

/* Perform LSD Test. */

PROC GLM DATA=A OUTSTAT=W;
 CLASS TRT;
 MODEL ARCSURV=TRT;
 MEANS TRT/LSD ALPHA=.1;
 TITLE3 'LSD TEST';

/* Perform t-tests for each dredged sediment-reference sediment comparison. */

DATA T1;
 SET A;
 IF TRT>2 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR ARCSURV;
 TITLE3 'T-TEST';
DATA T2;
 SET A;
 IF TRT=2 OR TRT=4 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR ARCSURV;
DATA T3;
 SET A;
 IF TRT=2 OR TRT=3 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR ARCSURV;

/* Test normality and equality of variances of rankits. */

PROC GLM NOPRINT DATA=A;
 CLASS TRT;
 MODEL RANKIT=TRT;
 OUTPUT OUT=Z1 R=RESID;
 TITLE2 'SURVIVAL DATA CONVERTED TO RANKITS';
PROC UNIVARIATE NORMAL;
 VAR RESID;
 TITLE3 'SHAPIRO-WILKS TEST FOR NORMALITY';
PROC MEANS DATA=A NOPRINT;
 BY TRT; VAR RANKIT;
 OUTPUT OUT=X1 MEAN=MEAN;
DATA AX1;
```

```
 MERGE A X1; BY TRT;
 ABSDEV=ABS(RANKIT-MEAN);
 LABEL ABSDEV='ABSOLUTE DEVIATIONS FROM MEAN';
PROC GLM;
 CLASS TRT;
 MODEL ABSDEV=TRT;
 TITLE3 'LEVENE''S TEST';

/* Perform LSD test on rankits. */

PROC GLM DATA=A;
 CLASS TRT;
 MODEL RANKIT=TRT;
 MEANS TRT/LSD ALPHA=.1;
 TITLE3 'LSD TEST ON RANKITS';

/* Perform t-tests comparing each dredged sediment with the reference */
/* using rankits. */

PROC TTEST DATA=T1;
 CLASS TRT;
 VAR RANKIT;
 TITLE3 'T-TEST ON RANKITS';
PROC TTEST DATA=T2;
 CLASS TRT;
 VAR RANKIT;
PROC TTEST DATA=T3;
 CLASS TRT;
 VAR RANKIT;


/* Calculate power of an LSD test to detect true population differences */
/* of 10, 20, 30, 40 and 50% below mean (arcsine-transformed) reference */
/* sediment survival. */

DATA C1;
 SET W;
 IF _TYPE_^='ERROR' THEN DELETE;
 MSE=SS/DF;
 KEEP MSE DF;
DATA C2;
 MERGE Y X;
 IF TRT^=1 THEN DELETE;
 MEANPCT=MEANSURV/M;
DATA C3;
 MERGE C1 C2;
 TALPHA=TINV(.95,DF);
 LABEL M='NO. OF ORGANISMS AT START OF TEST'
       N='NO. OF REPLICATES'
       MEANPCT='MEAN REFERENCE SURVIVAL'
       MSE='MEAN SQUARE ERROR'
       DF='DEGREES OF FREEDOM, DF'
       TALPHA='T VALUE FOR (1-ALPHA=0.95,DF)';
 TITLE2 'POWER OF LSD TO DETECT A TRUE POPULATION DIFFERENCE (D)';
 TITLE3 'FROM MEAN REFERENCE SURVIVAL USING ARCSINE TRANSFORMATION';
PROC PRINT LABEL NOOBS; VAR M N MEANPCT MSE DF TALPHA;
DATA C;
 SET C3;
 DO PCTDIFF=10 TO 50 BY 10;
  SEDSURV=MEANPCT-PCTDIFF/100;
  ARCSURV=ARSIN(SQRT(SEDSURV));
  ARCDIFF=MEAN-ARCSURV;
  TBETA=ARCDIFF*SQRT(N/(2*MSE))-TALPHA;
```

```
  POWER=PROBT(TBETA,DF);
  OUTPUT;
  END;
 LABEL PCTDIFF='% REDUCTION IN SURVIVAL FROM REFERENCE'

       SEDSURV='DREDGED SEDIMENT SURVIVAL'
       ARCSURV='ARCSINE DREDGED SEDIMENT SURVIVAL'
       ARCDIFF='D'
       TBETA='T VALUE FOR (1-BETA,DF)';
PROC PRINT LABEL;
 VAR PCTDIFF SEDSURV ARCSURV ARCDIFF TBETA POWER;
 TITLE;
```

D4.2.2 BENTOX.SAS Program Output

BENTHIC TOXICITY DATA

OBS	TREATMENT GROUP	REPLICATE	NO. OF ORGANISMS PER REPLICATE	NUMBER OF SURVIVORS	ARCSINE TRANSFORMATION	NORMALIZED RANK FOR SURVIVAL
1	REFERENCE	1	20	20	1.57080	1.46660
2	REFERENCE	2	20	20	1.57080	1.46660
3	REFERENCE	3	20	19	1.34528	0.83164
4	REFERENCE	4	20	19	1.34528	0.83164
5	REFERENCE	5	20	20	1.57080	1.46660
6	SEDIMENT 1	1	20	17	1.17310	0.25276
7	SEDIMENT 1	2	20	16	1.10715	-0.18775
8	SEDIMENT 1	3	20	18	1.24905	0.58946
9	SEDIMENT 1	4	20	17	1.17310	0.25276
10	SEDIMENT 1	5	20	15	1.04720	-0.51861
11	SEDIMENT 2	1	20	15	1.04720	-0.51861
12	SEDIMENT 2	2	20	16	1.10715	-0.18775
13	SEDIMENT 2	3	20	13	0.93774	-0.83164
14	SEDIMENT 2	4	20	17	1.17310	0.25276
15	SEDIMENT 2	5	20	11	0.83548	-1.40341
16	SEDIMENT 3	1	20	17	1.17310	0.25276
17	SEDIMENT 3	2	20	12	0.88608	-1.12814
18	SEDIMENT 3	3	20	10	0.78540	-1.86824
19	SEDIMENT 3	4	20	16	1.10715	-0.18775
20	SEDIMENT 3	5	20	13	0.93774	-0.83164

BENTHIC TOXICITY DATA

OBS	TREATMENT GROUP	N	TOTAL	MEAN SURVIVAL	SE
1	REFERENCE	5	98	19.6	0.24495
2	SEDIMENT 1	5	83	16.6	0.50990
3	SEDIMENT 2	5	72	14.4	1.07703
4	SEDIMENT 3	5	68	13.6	1.28841

BENTHIC TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION

OBS	TREATMENT GROUP	N	MEAN	VARIANCE	S	SE
1	REFERENCE	5	1.48059	0.015257	0.12352	0.055239
2	SEDIMENT 1	5	1.14992	0.005820	0.07629	0.034119
3	SEDIMENT 2	5	1.02013	0.018147	0.13471	0.060244
4	SEDIMENT 3	5	0.97789	0.025477	0.15962	0.071382

BENTHIC TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
SHAPIRO-WILKS TEST FOR NORMALITY

UNIVARIATE PROCEDURE

Variable=RESID

N	20		
W:Normal	0.945932	Prob<W	0.3217

BENTHIC TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
LEVENE'S TEST FOR EQUALITY OF VARIANCES

General Linear Models Procedure

Dependent Variable: ABSDEV ABSOLUTE DEVIATIONS FROM MEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.01373434	0.00457811	1.74	0.1985
Error	16	0.04201517	0.00262595		
Corrected Total	19	0.05574951			

BENTHIC TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
LSD TEST

General Linear Models Procedure

T tests (LSD) for variable: ARCSURV

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.1 df= 16 MSE= 0.016175
Critical Value of T= 1.75
Least Significant Difference= 0.1404

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	1.4806	5	REFERENCE
B	1.1499	5	SEDIMENT 1
B C B	1.0201	5	SEDIMENT 2
C C	0.9779	5	SEDIMENT 3

BENTHIC TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
T-TEST

TTEST PROCEDURE

Variable: ARCSURV ARCSINE TRANSFORMATION

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	1.48059096	0.12351878	0.05523928
SEDIMENT 1	5	1.14991717	0.07629145	0.03411857

Variances	T	DF	Prob>|T|
Unequal	5.0930	6.7	0.0017
Equal	5.0930	8.0	0.0009

For H0: Variances are equal, F' = 2.62 DF = (4,4) Prob>F' = 0.3733

BENTHIC TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
T-TEST

TTEST PROCEDURE

Variable: ARCSURV ARCSINE TRANSFORMATION

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	1.48059096	0.12351878	0.05523928
SEDIMENT 2	5	1.02013391	0.13470903	0.06024371

Variances	T	DF	Prob>|T|
Unequal	5.6335	7.9	0.0005
Equal	5.6335	8.0	0.0005

For H0: Variances are equal, F' = 1.19 DF = (4,4) Prob>F' = 0.8706

BENTHIC TOXICITY DATA
ARCSINE-SQUARE ROOT TRANSFORMATION
T-TEST

TTEST PROCEDURE

Variable: ARCSURV ARCSINE TRANSFORMATION

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	1.48059096	0.12351878	0.05523928
SEDIMENT 3	5	0.97789308	0.15961511	0.07138205

Variances	T	DF	Prob>|T|
Unequal	5.5695	7.5	0.0007
Equal	5.5695	8.0	0.0005

For H0: Variances are equal, F' = 1.67 DF = (4,4) Prob>F' = 0.6315

BENTHIC TOXICITY DATA
SURVIVAL DATA CONVERTED TO RANKITS
SHAPIRO-WILKS TEST FOR NORMALITY

UNIVARIATE PROCEDURE

Variable=RESID

N	20		
W:Normal	0.981773	Prob<W	0.9399

BENTHIC TOXICITY DATA
SURVIVAL DATA CONVERTED TO RANKITS
LEVENE'S TEST
General Linear Models Procedure

Dependent Variable: ABSDEV ABSOLUTE DEVIATIONS FROM MEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.31609842	0.10536614	1.18	0.3493
Error	16	1.43149144	0.08946821		
Corrected Total	19	1.74758986			

BENTHIC TOXICITY DATA
SURVIVAL DATA CONVERTED TO RANKITS
LSD TEST ON RANKITS

General Linear Models Procedure

T tests (LSD) for variable: RANKIT

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.1 df= 16 MSE= 0.346143
Critical Value of T= 1.75
Least Significant Difference= 0.6496

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	1.213	5	REFERENCE
B	0.078	5	SEDIMENT 1
B C B	-0.538	5	SEDIMENT 2
C C	-0.753	5	SEDIMENT 3

BENTHIC TOXICITY DATA
SURVIVAL DATA CONVERTED TO RANKITS
T-TEST ON RANKITS

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE SURV

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	1.21261524	0.34778201	0.15553284
SEDIMENT 1	5	0.07772091	0.43279236	0.19355063

Variances	T	DF	Prob>|T|
Unequal	4.5707	7.6	0.0021
Equal	4.5707	8.0	0.0018

For H0: Variances are equal, F' = 1.55 DF = (4,4) Prob>F' = 0.6821

BENTHIC TOXICITY DATA
SURVIVAL DATA CONVERTED TO RANKITS
T-TEST ON RANKITS

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE SURV

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	1.21261524	0.34778201	0.15553284
SEDIMENT 2	5	-0.53773198	0.62918751	0.28138121

Variances	T	DF	Prob>|T|
Unequal	5.4442	6.2	0.0014
Equal	5.4442	8.0	0.0006

For H0: Variances are equal, F' = 3.27 DF = (4,4) Prob>F' = 0.2773

BENTHIC TOXICITY DATA
SURVIVAL DATA CONVERTED TO RANKITS
T-TEST ON RANKITS

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE SURV

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	1.21261524	0.34778201	0.15553284
SEDIMENT 3	5	-0.75260418	0.82488344	0.36889909

Variances	T	DF	Prob>|T|
Unequal	4.9088	5.4	0.0038
Equal	4.9088	8.0	0.0012

For H0: Variances are equal, F' = 5.63 DF = (4,4) Prob>F' = 0.1229

BENTHIC TOXICITY DATA
POWER OF LSD TO DETECT A TRUE POPULATION DIFFERENCE (D)
FROM MEAN REFERENCE SURVIVAL USING ARCSINE TRANSFORMATION

NO. OF ORGANISMS AT START OF TEST	NO. OF REPLICATES	MEAN REFERENCE SURVIVAL	MEAN SQUARE ERROR	DEGREES OF FREEDOM, DF	T VALUE FOR (1-ALPHA=0.95,DF)
20	5	0.98	0.016175	16	1.74588

OBS	% REDUCTION IN SURVIVAL FROM REFERENCE	DREDGED SEDIMENT SURVIVAL	ARCSINE DREDGED SEDIMENT SURVIVAL	D	T VALUE FOR (1-BETA,DF)	POWER
1	10	0.88	1.21705	0.26354	1.53043	0.92728
2	20	0.78	1.08259	0.39800	3.20210	0.99722
3	30	0.68	0.96953	0.51106	4.60766	0.99985
4	40	0.58	0.86574	0.61485	5.89797	0.99999
5	50	0.48	0.76539	0.71520	7.14555	1.00000

D4.3 Program BIOACC.SAS for Single-Time Point Bioaccumulation Test Data Analysis

BIOACC.SAS is a program to compare Tier III bioaccumulation data from dredged sediments vs. reference sediment, using raw data and $\log_{10}$ transformation. Included in these analyses are: mean bioaccumulation from each sediment exposure, Shapiro-Wilk's Test for normality, Levene's Test for equality of variances, *t*-tests for equal or unequal variances, LSD test, and tests on rankits (normalized ranks for contaminant concentration). Refer to the decision tree in Figures D-5A and 5B to determine which test results should be used. The program includes power calculations for an LSD test on untransformed bioaccumulation data.

D4.3.1 BIOACC.SAS Program Statements

```
LIBNAME Q 'C:\SAS';
OPTIONS LINESIZE=79 PAGESIZE=59 NODATE NONUMBER;

/* Identify the treatment codes. */

PROC FORMAT;
 VALUE TRTFMT
   1='REFERENCE '
   2='SEDIMENT 1'
   3='SEDIMENT 2'
   4='SEDIMENT 3';

/* Input the bioaccumulation data after the CARDS statement, listing the */
/* treatment code, replicate, and contaminant concentration.  A permanent */
/* SAS data set is created in the directory specified in the LIBNAME */
/* statement. */
```

```
DATA Q.BIOACC;
 INPUT TRT REP CONC @@;
 CARDS;
1 1 .06 1 2 .05 1 3 .05 1 4 .08 1 5 .09
2 1 .16 2 2 .19 2 3 .18 2 4 .22 2 5 .31
3 1 .24 3 2 .10 3 3 .13 3 4 .18 3 5 .30
4 1 .13 4 2 .05 4 3 .17 4 4 .08 4 5 .22
;

/* Format, print, sort the data.  Print no. of observations, mean, and */
/* standard error for concentration in each treatment for both */
/* untransformed and log10-transformed data.  Calculate rankits. */

DATA A0;
 SET Q.BIOACC;
 LOGCONC=LOG10(CONC);
 MERGEVAR=1;
 LABEL TRT='TREATMENT GROUP'
       REP='REPLICATE'
       CONC='CONTAMINANT CONCENTRATION, ug/g'
       LOGCONC='LOG10 CONCENTRATION';
 FORMAT TRT TRTFMT.;
 TITLE 'SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA';
PROC RANK NORMAL=BLOM OUT=A;
 VAR CONC; RANKS RANKIT;
PROC PRINT LABEL; VAR TRT REP CONC LOGCONC RANKIT;
 LABEL RANKIT='NORMALIZED RANK FOR CONCENTRATION';
PROC SORT; BY TRT;
PROC MEANS NOPRINT; BY TRT; VAR CONC LOGCONC; ID MERGEVAR;
 OUTPUT OUT=Y N=N NLOG MEAN=MEANCONC MEANLOG VAR=S2 S2LOG STDERR=SE SELOG;
PROC PRINT LABEL; VAR TRT N MEANCONC S2 SE MEANLOG S2LOG SELOG;
 LABEL    MEANCONC='MEAN CONTAMINANT CONC.'
      S2='VARIANCE'
      SE='STANDARD ERROR'
      MEANLOG='MEAN LOG10 CONC.'
      S2LOG='VARIANCE OF LOGS'
      SELOG='STANDARD ERROR OF LOGS';

/* Test normality of residuals of untransformed and log-transformed data */
/* using Shapiro-Wilk's Test. */

PROC GLM NOPRINT DATA=A;
 CLASS TRT;
 MODEL CONC LOGCONC=TRT;
 OUTPUT OUT=Z R=RESID RESIDLOG;
PROC UNIVARIATE NORMAL;
 VAR RESID RESIDLOG;
 TITLE2 'SHAPIRO-WILKS TEST FOR NORMALITY';

/* Conduct Levene's Test for equality of variances of untransformed and */
/* log-transformed data. */

DATA AY;
 MERGE A Y; BY TRT;
 ABSDEV=ABS(CONC-MEANCONC);
 ABSLOG=ABS(LOGCONC-MEANLOG);
 LABEL    ABSDEV='ABSOLUTE DEVIATIONS FROM MEAN CONC.'
      ABSLOG='ABSOLUTE DEVIATIONS FROM MEAN LOGCONC.';
PROC GLM;
 CLASS TRT;
 MODEL ABSDEV ABSLOG=TRT;
 TITLE2 'LEVENE''S TEST';
/* Perform LSD on untransformed and log-transformed data. */
```

```
PROC GLM DATA=A OUTSTAT=W1;
 CLASS TRT;
 MODEL CONC=TRT;
 MEANS TRT/LSD ALPHA=.1;
 TITLE2 'LSD TEST (UNTRANSFORMED DATA)';
PROC GLM DATA=A OUTSTAT=W2;
 CLASS TRT;
 MODEL LOGCONC=TRT;
 MEANS TRT/LSD ALPHA=.1;
 TITLE2 'LSD TEST (LOG-TRANSFORMED DATA)';

/* Perform t-tests for each dredged sediment-reference sediment comparison */
/* using untransformed and log-transformed data. */

DATA T1;
 SET A;
 IF TRT>2 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR CONC LOGCONC;
 TITLE2 'T-TEST';
DATA T2;
 SET A;
 IF TRT=2 OR TRT=4 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR CONC LOGCONC;
DATA T3;
 SET A;
 IF TRT=2 OR TRT=3 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR CONC LOGCONC;

/* Test normality and equality of variances of rankits. */

PROC GLM NOPRINT DATA=A;
 CLASS TRT;
 MODEL RANKIT=TRT;
 OUTPUT OUT=Z2 R=RESID;
 TITLE2 'BIOACCUMULATION DATA CONVERTED TO RANKITS';
PROC UNIVARIATE NORMAL;
 VAR RESID;
 TITLE3 'SHAPIRO-WILKS TEST FOR NORMALITY';
PROC MEANS DATA=A NOPRINT;
 BY TRT; VAR RANKIT;
 OUTPUT OUT=X MEAN=MEAN;
DATA AX;
 MERGE A X; BY TRT;
 ABSDEV=ABS(RANKIT-MEAN);
PROC GLM;
 CLASS TRT;
 MODEL ABSDEV=TRT;
 TITLE3 'LEVENE''S TEST';

/* Perform LSD on rankits. */

PROC GLM DATA=A;
 CLASS TRT;
 MODEL RANKIT=TRT;
 MEANS TRT/LSD ALPHA=.1;
```

```
 TITLE3 'LSD TEST';

/* Perform t-tests for each dredged sediment-reference sediment comparison */
/* using rankits. */

PROC TTEST DATA=T1;
 CLASS TRT;
 VAR RANKIT;
 TITLE3 'T-TEST';
PROC TTEST DATA=T2;
 CLASS TRT;
 VAR RANKIT;
PROC TTEST DATA=T3;
 CLASS TRT;
 VAR RANKIT;

/* Calculate power of an LSD test to detect true population differences */
/* 10, 25, 50, and 100% above the reference mean contaminant concentration. */

DATA C1;
 SET W1;
 IF _TYPE_^='ERROR' THEN DELETE;
 MSE=SS/DF;
 MERGEVAR=1;
 KEEP MSE DF MERGEVAR;
DATA C2;
 SET Y;
 IF TRT^=1 THEN DELETE;
DATA C3;
 MERGE C1 C2;
 TALPHA=TINV(.95,DF);
 LABEL    N='NO. OF REPLICATES, N'
      MEANCONC='REFERENCE MEAN CONTAMINANT CONCENTRATION'
      MSE='MEAN SQUARE ERROR, MSE'
      DF='DEGREES OF FREEDOM, DF'
      TALPHA='T VALUE FOR (1-ALPHA=0.95,DF)';
 TITLE2 'POWER OF LSD TO DETECT A TRUE POPULATION DIFFERENCE (D)';
 TITLE3 'ABOVE REFERENCE MEAN CONTAMINANT CONCENTRATION';
PROC PRINT LABEL NOOBS; VAR N MEANCONC MSE DF TALPHA;
DATA C4;
 SET C3;
 DO PCTDIFF=10,25,50,100,200,300;
  SEDCONC=MEANCONC+((PCTDIFF/100)*MEANCONC);
  D=SEDCONC-MEANCONC;
  TBETA=D*SQRT(N/(2*MSE))-TALPHA;
  POWER=PROBT(TBETA,DF);
  OUTPUT;
  END;
 LABEL    PCTDIFF='% INCREASE IN CONC. ABOVE REFERENCE'
      SEDCONC='DREDGED SEDIMENT BIOACCUMULATION'
      TBETA='T VALUE FOR (1-BETA,DF)'
      POWER='POWER (1-BETA)';
PROC PRINT LABEL NOOBS; VAR PCTDIFF SEDCONC D TBETA POWER;
 TITLE 'POWER OF LSD TO DETECT % INCREASE IN CONCENTRATION ABOVE REFERENCE';
 TITLE2 'MEAN CONTAMINANT CONCENTRATION GIVEN N, MSE AND DF SHOWN ABOVE';
DATA C5;
 SET C3;
 DO POWER=.5,.6,.7,.8,.9,.95,.99;
  TBETA=TINV(POWER,DF);
  D=((TBETA+TALPHA)*SQRT(2*MSE))/SQRT(N);
  SEDCONC=MEANCONC+D;
  PCTDIFF=(D*100)/MEANCONC;
  OUTPUT;
```

```
  END;
 LABEL    SEDCONC='DREDGED SEDIMENT BIOACCUMULATION'
       PCTDIFF='% INCREASE IN CONC. ABOVE REFERENCE'
       TBETA='T VALUE FOR (1-BETA,DF)'
       POWER='POWER (1-BETA)';
PROC PRINT LABEL NOOBS; VAR POWER D SEDCONC PCTDIFF TBETA;
 TITLE 'MINIMUM DREDGED SEDIMENT BIOACCUMULATION THAT CAN BE DETECTED BY LSD';
 TITLE2 'AS SIGNIFICANT GIVEN SPECIFIED POWER AND N, MSE, AND DF SHOWN ABOVE';

/* Calculation of upper confidence limits (UCL) for comparison of mean */
/* dredged sediment bioaccumulation with an action level. */

DATA D;
 MERGE C1 Y; BY MERGEVAR;
 IF TRT=1 THEN DELETE;
 TALPHA1=TINV(.95,DF);
 TALPHA2=TINV(.95,N-1);
 UCL1=MEANCONC+TALPHA1*(SQRT(MSE/N));
 UCL2=MEANCONC+TALPHA2*(SQRT(S2/N));
 DMIN1=TALPHA1*SQRT(MSE/N);
 DMIN2=TALPHA2*SQRT(S2/N);
 LABEL    UCL1='UCL (EQUAL VARIANCES)'
       UCL2='UCL (UNEQUAL VARIANCES)'
       TALPHA1='T VALUE FOR (1-ALPHA=.95,DF)'
       TALPHA2='T VALUE FOR (1-ALPHA=.95,N-1)'
       DMIN1='MINIMUM SIGNIFICANT DIFFERENCE'
       DMIN2='MINIMUM SIGNIFICANT DIFFERENCE'
       MSE='MEAN SQUARE ERROR'
       S2='VARIANCE'
       MEANCONC='MEAN BIOACCUMULATION';
 TITLE 'COMPARISON OF MEAN DREDGED SEDIMENT BIOACCUMULATION WITH ACTION
LEVEL:';
PROC PRINT LABEL NOOBS; VAR TRT MEANCONC UCL1 MSE TALPHA1 DF DMIN1;
 TITLE2 'UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE EQUAL';
PROC PRINT LABEL NOOBS; VAR TRT MEANCONC UCL2 S2 TALPHA2 N DMIN2;
 TITLE2 'UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE UNEQUAL';


/* Calculate power of dredged sediment-action level comparisons using */
/* MSE given 10, 20, 30, 40, and 50% decreases in mean concentration */
/* below action level. */

DATA D1;
 SET C3;
 ACTION=.2;
 DO PCTDIFF=10 TO 50 BY 10;
  D=PCTDIFF*ACTION/100;
  SEDCONC=ACTION-D;
  TBETA=D*SQRT(N/MSE)-TALPHA;
  POWER=PROBT(TBETA,DF);
  OUTPUT;
  END;
 LABEL    PCTDIFF='% DECREASE BELOW ACTION LEVEL'
       SEDCONC='MEAN DREDGED SEDIMENT BIOACCUMULATION'
       TBETA='T VALUE FOR (1-BETA,DF)'
       POWER='POWER (1-BETA)';
PROC PRINT NOOBS LABEL; VAR PCTDIFF SEDCONC D TBETA POWER;
 TITLE 'POWER TO DETECT % DECREASE IN CONCENTRATION BELOW';
 TITLE2 'ACTION LEVEL OF 0.2 ug/g GIVEN N, MSE AND DF SHOWN ABOVE';
```

D4.3.2 BIOACC.SAS Program Output

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA

OBS	TREATMENT GROUP	REPLICATE	CONTAMINANT CONCENTRATION, ug/g	LOG10 CONCENTRATION	NORMALIZED RANK FOR CONCENTRATION
1	REFERENCE	1	0.06	-1.22185	-0.91914
2	REFERENCE	2	0.05	-1.30103	-1.46660
3	REFERENCE	3	0.05	-1.30103	-1.46660
4	REFERENCE	4	0.08	-1.09691	-0.66680
5	REFERENCE	5	0.09	-1.04576	-0.44777
6	SEDIMENT 1	1	0.16	-0.79588	0.06193
7	SEDIMENT 1	2	0.19	-0.72125	0.58946
8	SEDIMENT 1	3	0.18	-0.74473	0.38117
9	SEDIMENT 1	4	0.22	-0.65758	0.83164
10	SEDIMENT 1	5	0.31	-0.50864	1.86824
11	SEDIMENT 2	1	0.24	-0.61979	1.12814
12	SEDIMENT 2	2	0.10	-1.00000	-0.31457
13	SEDIMENT 2	3	0.13	-0.88606	-0.12434
14	SEDIMENT 2	4	0.18	-0.74473	0.38117
15	SEDIMENT 2	5	0.30	-0.52288	1.40341
16	SEDIMENT 3	1	0.13	-0.88606	-0.12434
17	SEDIMENT 3	2	0.05	-1.30103	-1.46660
18	SEDIMENT 3	3	0.17	-0.76955	0.18676
19	SEDIMENT 3	4	0.08	-1.09691	-0.66680
20	SEDIMENT 3	5	0.22	-0.65758	0.83164

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA

OBS	TREATMENT GROUP	N	MEAN CONTAMINANT CONC.	VARIANCE	STANDARD ERROR	MEAN LOG10 CONC.	VARIANCE OF LOGS	STANDARD ERROR OF LOGS
1	REFERENCE	5	0.066	.00033	0.008124	-1.19332	0.013772	0.05248
2	SEDIMENT 1	5	0.212	.00347	0.026344	-0.68561	0.012257	0.04951
3	SEDIMENT 2	5	0.190	.00660	0.036332	-0.75469	0.037367	0.08645
4	SEDIMENT 3	5	0.130	.00465	0.030496	-0.94223	0.066666	0.11547

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
SHAPIRO-WILKS TEST FOR NORMALITY

UNIVARIATE PROCEDURE

Variable=RESID

N	20		
W:Normal	0.957973	Prob<W	0.5111

Variable=RESIDLOG

N	20		
W:Normal	0.980207	Prob<W	0.9208

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
LEVENE'S TEST
General Linear Models Procedure

Dependent Variable: ABSDEV ABSOLUTE DEVIATIONS FROM MEAN CONC.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00647280	0.00215760	2.15	0.1339
Error	16	0.01605600	0.00100350		
Corrected Total	19	0.02252880			

Dependent Variable: ABSLOG ABSOLUTE DEVIATIONS FROM MEAN LOGCONC.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.04702396	0.01567465	2.19	0.1291
Error	16	0.11456390	0.00716024		
Corrected Total	19	0.16158786			

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
LSD TEST (UNTRANSFORMED DATA)

General Linear Models Procedure

T tests (LSD) for variable: CONC

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.1 df= 16 MSE= 0.003763
Critical Value of T= 1.75
Least Significant Difference= 0.0677

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	0.2120	5	SEDIMENT 1
A			
B A	0.1900	5	SEDIMENT 2
B			
B C	0.1300	5	SEDIMENT 3
C			
C	0.0660	5	REFERENCE

LSD TEST (LOG-TRANSFORMED DATA)

Alpha= 0.1 df= 16 MSE= 0.032515
Critical Value of T= 1.75
Least Significant Difference= 0.1991

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	-0.686	5	SEDIMENT 1
A			
B A	-0.755	5	SEDIMENT 2
B			
B	-0.942	5	SEDIMENT 3
C	-1.193	5	REFERENCE

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
T-TEST

TTEST PROCEDURE

Variable: CONC CONTAMINANT CONCENTRATION, ug/g

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	0.06600000	0.01816590	0.00812404
SEDIMENT 1	5	0.21200000	0.05890671	0.02634388

Variances	T	DF	Prob>|T|
Unequal	-5.2960	4.8	0.0039
Equal	-5.2960	8.0	0.0007

For H0: Variances are equal, F' = 10.52 DF = (4,4) Prob>F' = 0.0426

Variable: LOGCONC LOG10 CONCENTRATION

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-1.19331525	0.11735241	0.05248159
SEDIMENT 1	5	-0.68561391	0.11071260	0.04951218

Variances	T	DF	Prob>|T|
Unequal	-7.0366	8.0	0.0001
Equal	-7.0366	8.0	0.0001

For H0: Variances are equal, F' = 1.12 DF = (4,4) Prob>F' = 0.9128

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
T-TEST

TTEST PROCEDURE

Variable: CONC CONTAMINANT CONCENTRATION, ug/g

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	0.06600000	0.01816590	0.00812404
SEDIMENT 2	5	0.19000000	0.08124038	0.03633180

Variances	T	DF	Prob>|T|
Unequal	-3.3307	4.4	0.0258
Equal	-3.3307	8.0	0.0104

For H0: Variances are equal, F' = 20.00 DF = (4,4) Prob>F' = 0.0132

Variable: LOGCONC LOG10 CONCENTRATION

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-1.19331525	0.11735241	0.05248159
SEDIMENT 2	5	-0.75469033	0.19330562	0.08644890

Variances	T	DF	Prob>|T|
Unequal	-4.3371	6.6	0.0040
Equal	-4.3371	8.0	0.0025

For H0: Variances are equal, F' = 2.71 DF = (4,4) Prob>F' = 0.3570

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
T-TEST

TTEST PROCEDURE

Variable: CONC CONTAMINANT CONCENTRATION, ug/g

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	0.06600000	0.01816590	0.00812404
SEDIMENT 3	5	0.13000000	0.06819091	0.03049590

Variances	T	DF	Prob>|T|
Unequal	-2.0279	4.6	0.1045
Equal	-2.0279	8.0	0.0771

For H0: Variances are equal, F' = 14.09 DF = (4,4) Prob>F' = 0.0252

Variable: LOGCONC LOG10 CONCENTRATION

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-1.19331525	0.11735241	0.05248159
SEDIMENT 3	5	-0.94222501	0.25819757	0.11546947

Variances	T	DF	Prob>|T|
Unequal	-1.9796	5.6	0.0990
Equal	-1.9796	8.0	0.0831

For H0: Variances are equal, F' = 4.84 DF = (4,4) Prob>F' = 0.1558

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
BIOACCUMULATION DATA CONVERTED TO RANKITS
SHAPIRO-WILKS TEST FOR NORMALITY

UNIVARIATE PROCEDURE

Variable=RESID

N	20		
W:Normal	0.972308	Prob<W	0.7907

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
BIOACCUMULATION DATA CONVERTED TO RANKITS
LEVENE'S TEST

General Linear Models Procedure

Dependent Variable: ABSDEV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.24147324	0.08049108	0.61	0.6212
Error	16	2.12865866	0.13304117		
Corrected Total	19	2.37013190			

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
BIOACCUMULATION DATA CONVERTED TO RANKITS
LSD TEST

General Linear Models Procedure

T tests (LSD) for variable: RANKIT

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.1 df= 16 MSE= 0.503649
Critical Value of T= 1.75
Least Significant Difference= 0.7836

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	0.746	5	SEDIMENT 1
A			
B A	0.495	5	SEDIMENT 2
B			
B C	-0.248	5	SEDIMENT 3
C			
C	-0.993	5	REFERENCE

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
BIOACCUMULATION DATA CONVERTED TO RANKITS

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE CONC

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.99338019	0.46306944	0.20709095
SEDIMENT 1	5	0.74648762	0.68780736	0.30759680

Variances	T	DF	Prob>|T|
Unequal	-4.6920	7.0	0.0022
Equal	-4.6920	8.0	0.0016

For H0: Variances are equal, F' = 2.21 DF = (4,4) Prob>F' = 0.4623

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
BIOACCUMULATION DATA CONVERTED TO RANKITS

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE CONC

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.99338019	0.46306944	0.20709095
SEDIMENT 2	5	0.49476200	0.75465812	0.33749337

Variances	T	DF	Prob>|T|
Unequal	-3.7583	6.6	0.0079
Equal	-3.7583	8.0	0.0056

For H0: Variances are equal, F' = 2.66 DF = (4,4) Prob>F' = 0.3671

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
BIOACCUMULATION DATA CONVERTED TO RANKITS

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE CONC

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.99338019	0.46306944	0.20709095
SEDIMENT 3	5	-0.24786944	0.87038805	0.38924937

Variances	T	DF	Prob>|T|
Unequal	-1.6908	6.1	0.1411
Equal	-1.6908	8.0	0.1293

For H0: Variances are equal, F' = 3.53 DF = (4,4) Prob>F' = 0.2491

SINGLE-TIME POINT CONTAMINANT BIOACCUMULATION DATA
POWER OF LSD TO DETECT A TRUE POPULATION DIFFERENCE (D)
ABOVE REFERENCE MEAN CONTAMINANT CONCENTRATION

NO. OF REPLICATES, N	REFERENCE MEAN CONTAMINANT CONCENTRATION	MEAN SQUARE ERROR, MSE	DEGREES OF FREEDOM, DF	T VALUE FOR (1-ALPHA=0.95,DF)
5	0.066	.0037625	16	1.74588

POWER OF LSD TO DETECT % INCREASE IN CONCENTRATION ABOVE REFERENCE
MEAN CONTAMINANT CONCENTRATION GIVEN N, MSE AND DF SHOWN ABOVE

% INCREASE IN CONC. ABOVE REFERENCE	DREDGED SEDIMENT BIOACCUMULATION	D	T VALUE FOR (1-BETA,DF)	POWER (1-BETA)
10	0.0726	0.0066	-1.57576	0.06732
25	0.0825	0.0165	-1.32056	0.10261
50	0.0990	0.0330	-0.89524	0.19196
100	0.1320	0.0660	-0.04460	0.48249
200	0.1980	0.1320	1.65668	0.94147
300	0.2640	0.1980	3.35796	0.99800

MINIMUM DREDGED SEDIMENT BIOACCUMULATION THAT CAN BE DETECTED BY LSD
AS SIGNIFICANT GIVEN SPECIFIED POWER AND N, MSE, AND DF SHOWN ABOVE

POWER (1-BETA)	D	DREDGED SEDIMENT BIOACCUMULATION	% INCREASE IN CONC. ABOVE REFERENCE	T VALUE FOR (1-BETA,DF)
0.50	0.06773	0.13373	102.622	0.00000
0.60	0.07772	0.14372	117.763	0.25760
0.70	0.08849	0.15449	134.069	0.53501
0.80	0.10127	0.16727	153.446	0.86467
0.90	0.11959	0.18559	181.195	1.33676
0.95	0.13546	0.20146	205.244	1.74588
0.99	0.16796	0.23396	254.477	2.58349

COMPARISON OF MEAN DREDGED SEDIMENT BIOACCUMULATION WITH ACTION LEVEL:
UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE EQUAL

TREATMENT GROUP	MEAN BIOACCUMULATION	UCL (EQUAL VARIANCES)	MEAN SQUARE ERROR	T VALUE FOR (1-ALPHA=.95,DF)	DF	MINIMUM SIGNIFICANT DIFFERENCE
SEDIMENT 1	0.212	0.25989	.0037625	1.74588	16	0.047893
SEDIMENT 2	0.190	0.23789	.0037625	1.74588	16	0.047893
SEDIMENT 3	0.130	0.17789	.0037625	1.74588	16	0.047893

COMPARISON OF MEAN DREDGED SEDIMENT BIOACCUMULATION WITH ACTION LEVEL:
UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE UNEQUAL

TREATMENT GROUP	MEAN BIOACCUMULATION	UCL (UNEQUAL VARIANCES)	VARIANCE	T VALUE FOR (1-ALPHA=.95,N-1)	N	MINIMUM SIGNIFICANT DIFFERENCE
SEDIMENT 1	0.212	0.26816	.00347	2.13185	5	0.056161
SEDIMENT 2	0.190	0.26745	.00660	2.13185	5	0.077454
SEDIMENT 3	0.130	0.19501	.00465	2.13185	5	0.065013

POWER TO DETECT % DECREASE IN CONCENTRATION BELOW
ACTION LEVEL OF 0.2 ug/g GIVEN N, MSE AND DF SHOWN ABOVE

% DECREASE BELOW ACTION LEVEL	MEAN DREDGED SEDIMENT BIOACCUMULATION	D	T VALUE FOR (1-BETA,DF)	POWER (1-BETA)
10	0.18	0.02	-1.01680	0.16219
20	0.16	0.04	-0.28772	0.38863
30	0.14	0.06	0.44136	0.66757
40	0.12	0.08	1.17045	0.87052
50	0.10	0.10	1.89953	0.96216

D4.4 Program BIOACCSS.SAS for Time-Sequenced Bioaccumulation Test Data Analysis

BIOACCSS.SAS is a program to compare Tier IV estimated steady-state bioaccumulation (C_{ss}) from dredged sediments vs. reference sediment, using untransformed data and $\log_{10}$ transformation. Included are: data plots, estimation of C_{ss}, mean C_{ss} from each sediment exposure, Shapiro-Wilk's test for normality, Levene's test for equality of variances, LSD test, *t*-tests for equal or unequal variances, and tests on rankits (normalized ranks for C_{ss}). Refer to the decision tree in Figures D-5A and 5B to determine which test results should be used. The program includes power calculations for an LSD test on untransformed C_{ss} estimates.

D4.4.1 BIOACCSS.SAS Program Statements

```
LIBNAME Q 'C:\SAS';
OPTIONS LINESIZE=79 PAGESIZE=59 NONUMBER NODATE;

/* Identify the treatment codes. */

PROC FORMAT;
 VALUE TRTFMT
   1='REFERENCE '
   2='SEDIMENT 1'
   3='SEDIMENT 2'
   4='SEDIMENT 3';

/* Input the bioaccumulation data after the CARDS statement, listing the */
/* day, replicate, treatment code, and contaminant concentration.  A */
/* permanent SAS data set is created in the directory specified in the */
/* LIBNAME statement. */


DATA Q.BIOACCSS;
 INPUT DAY REP TRT CONC @@;
 CARDS;
2 1 1 .054 2 2 1 .163 2 3 1 .391 2 4 1 .234 2 5 1 .034
2 1 2 .159 2 2 2 .292 2 3 2 .428 2 4 2 .558 2 5 2 .256
2 1 3 .869 2 2 3 .726 2 3 3 .394 2 4 3 1.232 2 5 3 .977
2 1 4 .745 2 2 4 1.703 2 3 4 2.045 2 4 4 1.855 2 5 4 1.135
4 1 1 .441 4 2 1 .797 4 3 1 .203 4 4 1 .564 4 5 1 .018
4 1 2 .516 4 2 2 .158 4 3 2 .743 4 4 2 .324 4 5 2 .126
4 1 3 .838 4 2 3 .633 4 3 3 .452 4 4 3 .728 4 5 3 1.314
4 1 4 1.316 4 2 4 .930 4 3 4 2.141 4 4 4 1.150 4 5 4 1.621
7 1 1 .687 7 2 1 .177 7 3 1 .862 7 4 1 .413 7 5 1 .029
7 1 2 .881 7 2 2 .317 7 3 2 .270 7 4 2 .562 7 5 2 .603
7 1 3 1.246 7 2 3 .816 7 3 3 .897 7 4 3 1.639 7 5 3 .688
7 1 4 1.583 7 2 4 2.715 7 3 4 1.016 7 4 4 2.221 7 5 4 2.134
10 1 1 .037 10 2 1 .549 10 3 1 .884 10 4 1 .787 10 5 1 .294
10 1 2 .278 10 2 2 .485 10 3 2 .051 10 4 2 .909 10 5 2 .718
10 1 3 1.767 10 2 3 1.272 10 3 3 1.003 10 4 3 1.158 10 5 3 1.415
10 1 4 1.578 10 2 4 2.268 10 3 4 1.756 10 4 4 2.899 10 5 4 .890
18 1 1 .856 18 2 1 .598 18 3 1 .016 18 4 1 .806 18 5 1 .119
18 1 2 .904 18 2 2 1.300 18 3 2 .671 18 4 2 .934 18 5 2 1.173
18 1 3 1.631 18 2 3 1.877 18 3 3 1.487 18 4 3 1.216 18 5 3 1.280
18 1 4 2.822 18 2 4 2.607 18 3 4 3.414 18 4 4 1.319 18 5 4 1.866
28 1 1 .514 28 2 1 .839 28 3 1 .793 28 4 1 .899 28 5 1 .226
28 1 2 .172 28 2 2 1.049 28 3 2 .476 28 4 2 .712 28 5 2 1.245
28 1 3 1.178 28 2 3 1.721 28 3 3 1.366 28 4 3 1.513 28 5 3 1.843
28 1 4 1.295 28 2 4 2.964 28 3 4 2.109 28 4 4 2.820 28 5 4 3.325
;

/* Specify contaminant concentrations in the sediments.  Format, sort, */
/* and print the data. */

DATA AA;
 SET Q.BIOACCSS;
 SELECT (TRT);
  WHEN (1) CS=.45;
  WHEN (2) CS=4;
  WHEN (3) CS=33;
  WHEN (4) CS=44;
  OTHERWISE;
 END;
 LABEL TRT='TREATMENT GROUP'
```

```
      REP='REPLICATE'
      CONC='CONC. IN TISSUE'
      CS='CONC. IN SEDIMENT';
 FORMAT TRT TRTFMT.;
 TITLE 'TIME-SEQUENCED BIOACCUMULATION';
PROC SORT; BY TRT REP;
PROC PRINT LABEL; BY TRT; VAR REP DAY CONC CS;

/* Plot the data by treatment group, identifying the replicates.  Plots */
/* may be sent to the screen using the first GOPTIONS statement, or to a */
/* printer using the second GOPTIONS statement.  Consult the SAS/GRAPH */
/* User's Guide (SAS Institute, Inc., 1988c) for appropriate device names */
/* and instructions for GACCESS=. */

*GOPTIONS DEVICE=VGA;
GOPTIONS DEVICE=HPLJ3P GACCESS='SASGASTD>LPT2:' VSIZE=6 IN HSIZE=6.5 IN
   VORIGIN=3 IN HORIGIN=0.3 IN;
PROC GPLOT UNIFORM; BY TRT;
 PLOT CONC*DAY=REP;

/* Perform nonlinear regressions on each treatment and replicate. */
/* If you wish to use a method other than DUD, include the following */
/* derivative statements after the MODEL statement:  DER.K1=CS/K2*(1-EX); */
/* and DER.K2=CS*(K1/K2)*(DAY*EX-(1-EX)/K2);.  Save regression parameters */
/* in a permanent SAS data set. */

PROC NLIN BEST=10 METHOD=DUD;
 BY TRT REP;
 PARMS K1=0 TO 3 BY .1 K2=.01 TO 2 BY .1;
 EX=EXP(-K2*DAY);
 MODEL CONC=CS*(K1/K2)*(1-EX);
 OUTPUT OUT=Q.REGPARMS PARMS=K1 K2;

/* Calculate and print Css and regression parameters.  Log-transform Css. */
/* Calculate rankits.  Save these variables in a permanent SAS data set. */

DATA A;
 SET Q.REGPARMS;
 IF DAY<28 THEN DELETE;
 CSS=CS*K1/K2;
 LOGCSS=LOG10(CSS);
 DROP DAY CONC;
 LABEL    CSS='STEADY STATE CONC., Css'
      LOGCSS='Log10 Css'
      K1='UPTAKE RATE CONSTANT, k1'
      K2='DEPURATION RATE CONSTANT, k2';
 MERGEVAR=1;

PROC RANK NORMAL=BLOM OUT=Q.CSS;
 VAR CSS; RANKS RANKIT;
PROC PRINT LABEL DATA=Q.CSS; VAR TRT REP K1 K2 CSS LOGCSS RANKIT;
 LABEL      RANKIT='NORMALIZED RANK FOR Css';

/* Calculate and print descriptive statistics for Css and logCss. */

PROC MEANS NOPRINT DATA=Q.CSS; BY TRT; VAR CSS LOGCSS; ID MERGEVAR;
 OUTPUT OUT=Y N=N NLOG MEAN=MEANCSS MEANLOG VAR=S2 S2LOG STDERR=SE SELOG;
PROC PRINT LABEL; VAR TRT N MEANCSS S2 SE MEANLOG S2LOG SELOG;
 LABEL    MEANCSS='MEAN Css'
      S2='VARIANCE'
      SE='STANDARD ERROR'
      MEANLOG='MEAN Log10 Css'
      S2LOG='VARIANCE OF LOGS'
```

```
      SELOG='STANDARD ERROR OF LOGS';

/* Test normality of residuals of untransformed and log-transformed Css */
/* using Shapiro-Wilk's Test. */

PROC GLM NOPRINT DATA=Q.CSS;
 CLASS TRT;
 MODEL CSS LOGCSS=TRT;
 OUTPUT OUT=Z R=RESID RESIDLOG;
PROC UNIVARIATE NORMAL;
 VAR RESID RESIDLOG;
 TITLE2 'SHAPIRO-WILKS TEST FOR NORMALITY';
/* Conduct Levene's Test for equality of variances of untransformed and */
/* log-transformed Css. */

DATA AX;
 MERGE Q.CSS Y; BY TRT;
 ABSDEV=ABS(CSS-MEANCSS);
 ABSLOG=ABS(LOGCSS-MEANLOG);
 LABEL    ABSDEV='ABSOLUTE DEVIATIONS FROM Css MEAN'
      ABSLOG='ABSOLUTE DEVIATIONS FROM logCss MEAN';
PROC GLM;
 CLASS TRT;
 MODEL ABSDEV ABSLOG=TRT;
 TITLE2 'LEVENE''S TEST';

/* Perform LSD on untransformed and log-transformed Css. */

PROC GLM DATA=Q.CSS OUTSTAT=W1;
 CLASS TRT;
 MODEL CSS=TRT;
 MEANS TRT/LSD ALPHA=.1;
 TITLE2 'LSD TEST (UNTRANSFORMED DATA)';
PROC GLM DATA=Q.CSS OUTSTAT=W2;
 CLASS TRT;
 MODEL LOGCSS=TRT;
 MEANS TRT/LSD ALPHA=.1;
 TITLE2 'LSD TEST (LOG-TRANSFORMED DATA)';

/* Perform t-tests for each dredged sediment-reference sediment comparison */
/* using untransformed and log-transformed Css. */

DATA T1;
 SET Q.CSS;
 IF TRT>2 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR CSS LOGCSS;
 TITLE2 'T-TEST';
DATA T2;
 SET Q.CSS;
 IF TRT=2 OR TRT=4 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR CSS LOGCSS;
DATA T3;
 SET Q.CSS;
 IF TRT=2 OR TRT=3 THEN DELETE;
PROC TTEST;
 CLASS TRT;
 VAR CSS LOGCSS;

/* Test normality and equality of variances of rankits. */
```

```
PROC GLM NOPRINT DATA=Q.CSS;
 CLASS TRT;
 MODEL RANKIT=TRT;
 OUTPUT OUT=Z1 R=RESID;
 TITLE2 'Css CONVERTED TO RANKITS';
PROC UNIVARIATE NORMAL;
 VAR RESID;
 TITLE3 'SHAPIRO-WILKS TEST FOR NORMALITY';
PROC MEANS DATA=Q.CSS NOPRINT;
 BY TRT; VAR RANKIT;
 OUTPUT OUT=X2 MEAN=MEAN;
DATA AX2;
 MERGE Q.CSS X2; BY TRT;
 ABSDEV=ABS(RANKIT-MEAN);
PROC GLM;
 CLASS TRT;
 MODEL ABSDEV=TRT;
 TITLE3 'LEVENE''S TEST';

/* Perform LSD on rankits. */

PROC GLM DATA=Q.CSS;
 CLASS TRT;
 MODEL RANKIT=TRT;
 MEANS TRT/LSD ALPHA=.1;
 TITLE3 'LSD TEST';

/* Perform t-tests for each dredged sediment-reference sediment comparison */
/* using rankits. */

PROC TTEST DATA=T1;
 CLASS TRT; VAR RANKIT;
 TITLE3 'T-TEST';
PROC TTEST DATA=T2;
 CLASS TRT; VAR RANKIT;
PROC TTEST DATA=T3;
 CLASS TRT; VAR RANKIT;

/* Calculate power of an LSD test to detect true population differences */
/* 10, 25, 50, and 100% above the reference mean Css. */

DATA C1;
 SET W1;
 IF _TYPE_^='ERROR' THEN DELETE;
 MSE=SS/DF;
 MERGEVAR=1;
 KEEP MSE DF MERGEVAR;
DATA C2;
 SET Y;
 IF TRT^=1 THEN DELETE;
DATA C3;
 MERGE C1 C2;
 TALPHA=TINV(.95,DF);
 LABEL    N='NO. OF REPLICATES, N'
      MEANCSS='REFERENCE MEAN Css'
      MSE='MEAN SQUARE ERROR, MSE'
      DF='DEGREES OF FREEDOM, DF'
      TALPHA='T VALUE FOR (1-ALPHA=0.95,DF)';
 TITLE2 'POWER OF LSD TO DETECT A TRUE POPULATION DIFFERENCE (D)';
 TITLE3 'ABOVE REFERENCE MEAN Css';
PROC PRINT LABEL NOOBS; VAR N MEANCSS MSE DF TALPHA;
DATA C4;
 SET C3;
```

```
 DO PCTDIFF=10,25,50,100,200,300;
  SEDCSS=MEANCSS+((PCTDIFF/100)*MEANCSS);
  D=SEDCSS-MEANCSS;
  TBETA=D*SQRT(N/(2*MSE))-TALPHA;
  POWER=PROBT(TBETA,DF);
  OUTPUT;
  END;
 LABEL    PCTDIFF='% INCREASE IN Css ABOVE REFERENCE'
      SEDCSS='DREDGED SEDIMENT Css'
      TBETA='T VALUE FOR (1-BETA,DF)'
      POWER='POWER (1-BETA)';
PROC PRINT LABEL NOOBS; VAR PCTDIFF SEDCSS D TBETA POWER;
 TITLE 'POWER OF LSD TO DETECT % INCREASE IN Css ABOVE REFERENCE';
 TITLE2 'MEAN Css GIVEN N, MSE AND DF SHOWN ABOVE';
DATA C5;
 SET C3;
 DO POWER=.5,.6,.7,.8,.9,.95,.99;
  TBETA=TINV(POWER,DF);
  D=((TBETA+TALPHA)*SQRT(2*MSE))/SQRT(N);
  SEDCSS=MEANCSS+D;
  PCTDIFF=(D*100)/MEANCSS;
  OUTPUT;
  END;
 LABEL    SEDCSS='DREDGED SEDIMENT Css'
      PCTDIFF='% INCREASE IN Css ABOVE REFERENCE'
      TBETA='T VALUE FOR (1-BETA,DF)'
      POWER='POWER (1-BETA)';
PROC PRINT LABEL NOOBS; VAR POWER D SEDCSS PCTDIFF TBETA;
 TITLE 'MINIMUM DREDGED SEDIMENT Css THAT CAN BE DETECTED BY LSD';
 TITLE2 'AS SIGNIFICANT GIVEN SPECIFIED POWER AND N, MSE, AND DF SHOWN ABOVE';

/* Calculation of upper confidence limits (UCL) for comparison of mean */
/* dredged sediment Css with an action level. */

DATA D;
 MERGE C1 Y; BY MERGEVAR;
 IF TRT=1 THEN DELETE;
 TALPHA1=TINV(.95,DF);
 TALPHA2=TINV(.95,N-1);
 UCL1=MEANCSS+TALPHA1*(SQRT(MSE/N));
 UCL2=MEANCSS+TALPHA2*(SQRT(S2/N));
 DMIN1=TALPHA1*SQRT(MSE/N);
 DMIN2=TALPHA2*SQRT(S2/N);
 LABEL    UCL1='UCL (EQUAL VARIANCES)'
      UCL2='UCL (UNEQUAL VARIANCES)'
      TALPHA1='T VALUE FOR (1-ALPHA=.95,DF)'
      TALPHA2='T VALUE FOR (1-ALPHA=.95,N-1)'
      DMIN1='MINIMUM SIGNIFICANT DIFFERENCE'
      DMIN2='MINIMUM SIGNIFICANT DIFFERENCE'
      MSE='MEAN SQUARE ERROR'
      S2='VARIANCE'
      MEANCSS='MEAN DREDGED SEDIMENT Css';
 TITLE 'COMPARISON OF MEAN DREDGED SEDIMENT Css WITH ACTION LEVEL:';
PROC PRINT LABEL NOOBS; VAR TRT MEANCSS UCL1 MSE TALPHA1 DF DMIN1;
 TITLE2 'UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE EQUAL';
PROC PRINT LABEL NOOBS; VAR TRT MEANCSS UCL2 S2 TALPHA2 N DMIN2;
 TITLE2 'UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE UNEQUAL';

/* Calculate power of dredged sediment-action level comparisons using */
/* MSE given 10, 20, 30, 40, and 50% decreases in mean Css below */
/* action level. */
```

```
DATA D1;
 SET C3;
 ACTION=2;
 DO PCTDIFF=10 TO 50 BY 10;
  D=PCTDIFF*ACTION/100;
  SEDCSS=ACTION-D;
  TBETA=D*SQRT(N/MSE)-TALPHA;
  POWER=PROBT(TBETA,DF);
  OUTPUT;
  END;
 LABEL    PCTDIFF='% DECREASE BELOW ACTION LEVEL'
      SEDCSS='DREDGED SEDIMENT Css'
      TBETA='T VALUE FOR (1-BETA,DF)'
      POWER='POWER (1-BETA)';
PROC PRINT NOOBS LABEL; VAR PCTDIFF SEDCSS D TBETA POWER;
 TITLE 'POWER TO DETECT % DECREASE IN Css BELOW';
 TITLE2 'ACTION LEVEL OF 2 ug/g GIVEN N, MSE AND DF SHOWN ABOVE';
```

D4.4.2 BIOACCSS.SAS Program Output

TIME-SEQUENCED BIOACCUMULATION

---------- TREATMENT GROUP=REFERENCE ----------

OBS	REPLICATE	DAY	CONC. IN TISSUE	CONC. IN SEDIMENT
1	1	2	0.054	0.45
2	1	4	0.441	0.45
3	1	7	0.687	0.45
4	1	10	0.037	0.45
5	1	18	0.856	0.45
6	1	28	0.514	0.45
7	2	2	0.163	0.45
8	2	4	0.797	0.45
9	2	7	0.177	0.45
10	2	10	0.549	0.45
11	2	18	0.598	0.45
12	2	28	0.839	0.45
13	3	2	0.391	0.45
14	3	4	0.203	0.45
15	3	7	0.862	0.45
16	3	10	0.884	0.45
17	3	18	0.016	0.45
18	3	28	0.793	0.45
19	4	2	0.234	0.45
20	4	4	0.564	0.45
21	4	7	0.413	0.45
22	4	10	0.787	0.45
23	4	18	0.806	0.45
24	4	28	0.899	0.45
25	5	2	0.034	0.45
26	5	4	0.018	0.45
27	5	7	0.029	0.45
28	5	10	0.294	0.45
29	5	18	0.119	0.45
30	5	28	0.226	0.45

---------- TREATMENT GROUP=SEDIMENT 1 ----------

31	1	2	0.159	4
32	1	4	0.516	4
33	1	7	0.881	4
34	1	10	0.278	4
35	1	18	0.904	4
36	1	28	0.172	4
37	2	2	0.292	4
38	2	4	0.158	4
39	2	7	0.317	4
40	2	10	0.485	4
41	2	18	1.300	4
42	2	28	1.049	4
43	3	2	0.428	4
44	3	4	0.743	4
45	3	7	0.270	4
46	3	10	0.051	4
47	3	18	0.671	4
48	3	28	0.476	4
49	4	2	0.558	4
50	4	4	0.324	4
51	4	7	0.562	4
52	4	10	0.909	4
53	4	18	0.934	4
54	4	28	0.712	4
55	5	2	0.256	4
56	5	4	0.126	4
57	5	7	0.603	4
58	5	10	0.718	4
59	5	18	1.173	4
60	5	28	1.245	4

---------- TREATMENT GROUP=SEDIMENT 2 ----------

61	1	2	0.869	33
62	1	4	0.838	33
63	1	7	1.246	33
64	1	10	1.767	33
65	1	18	1.631	33
66	1	28	1.178	33
67	2	2	0.726	33
68	2	4	0.633	33
69	2	7	0.816	33
70	2	10	1.272	33
71	2	18	1.877	33
72	2	28	1.721	33
73	3	2	0.394	33
74	3	4	0.452	33
75	3	7	0.897	33
76	3	10	1.003	33
77	3	18	1.487	33
78	3	28	1.366	33
79	4	2	1.232	33
80	4	4	0.728	33
81	4	7	1.639	33

TIME-SEQUENCED BIOACCUMULATION

82	4	10	1.158	33
83	4	18	1.216	33
84	4	28	1.513	33
85	5	2	0.977	33
86	5	4	1.314	33
87	5	7	0.688	33
88	5	10	1.415	33
89	5	18	1.280	33
90	5	28	1.843	33

---------- TREATMENT GROUP=SEDIMENT 3 ----------

91	1	2	0.745	44
92	1	4	1.316	44
93	1	7	1.583	44
94	1	10	1.578	44
95	1	18	2.822	44
96	1	28	1.295	44
97	2	2	1.703	44
98	2	4	0.930	44
99	2	7	2.715	44
100	2	10	2.268	44
101	2	18	2.607	44
102	2	28	2.964	44
103	3	2	2.045	44
104	3	4	2.141	44
105	3	7	1.016	44
106	3	10	1.756	44
107	3	18	3.414	44
108	3	28	2.109	44
109	4	2	1.855	44
110	4	4	1.150	44
111	4	7	2.221	44
112	4	10	2.899	44
113	4	18	1.319	44
114	4	28	2.820	44
115	5	2	1.135	44
116	5	4	1.621	44
117	5	7	2.134	44
118	5	10	0.890	44
119	5	18	1.866	44

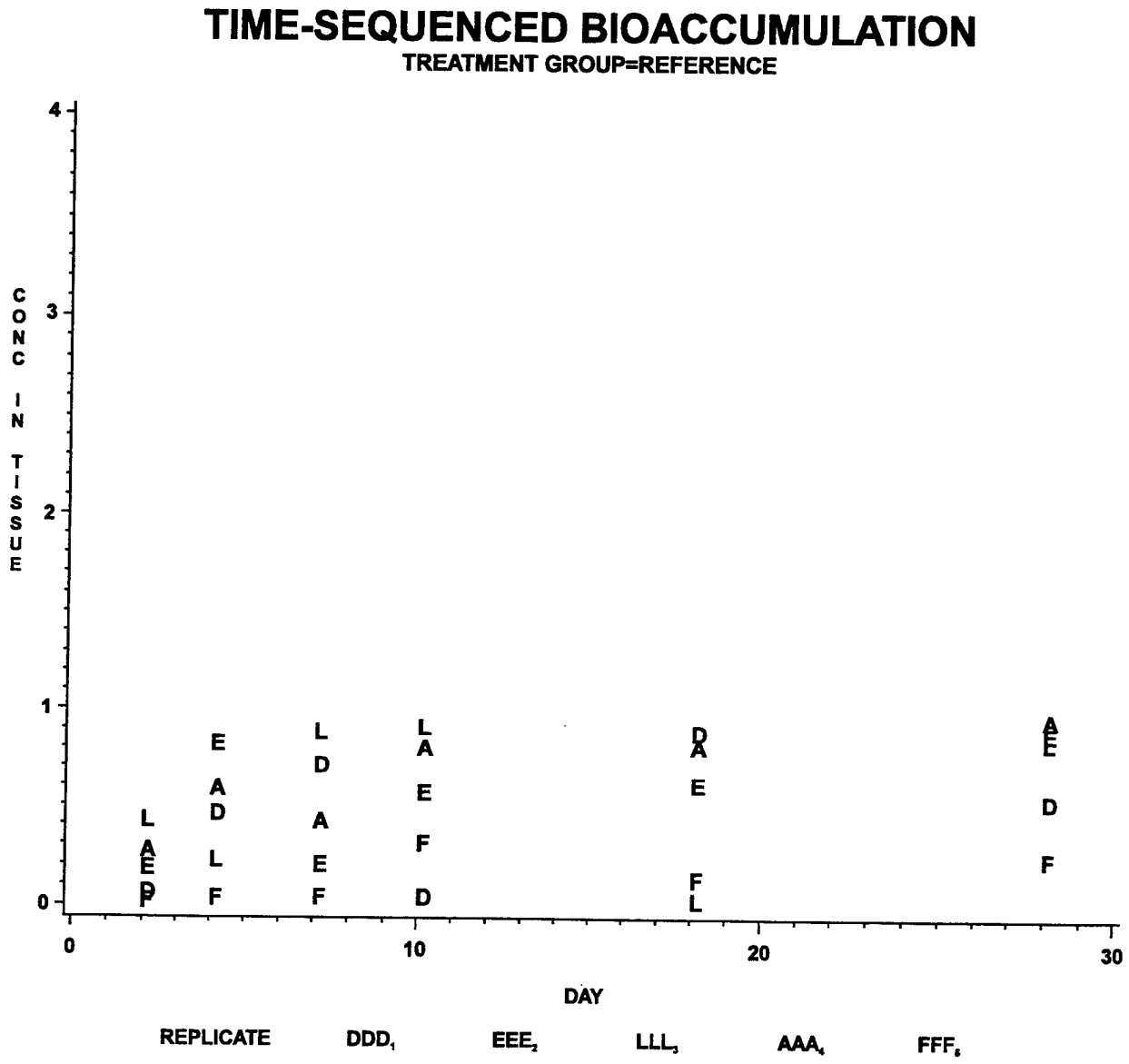


Figure D-8. Plot of Time-Sequenced Bioaccumulation Reference Sediment Example Data by Replicate.

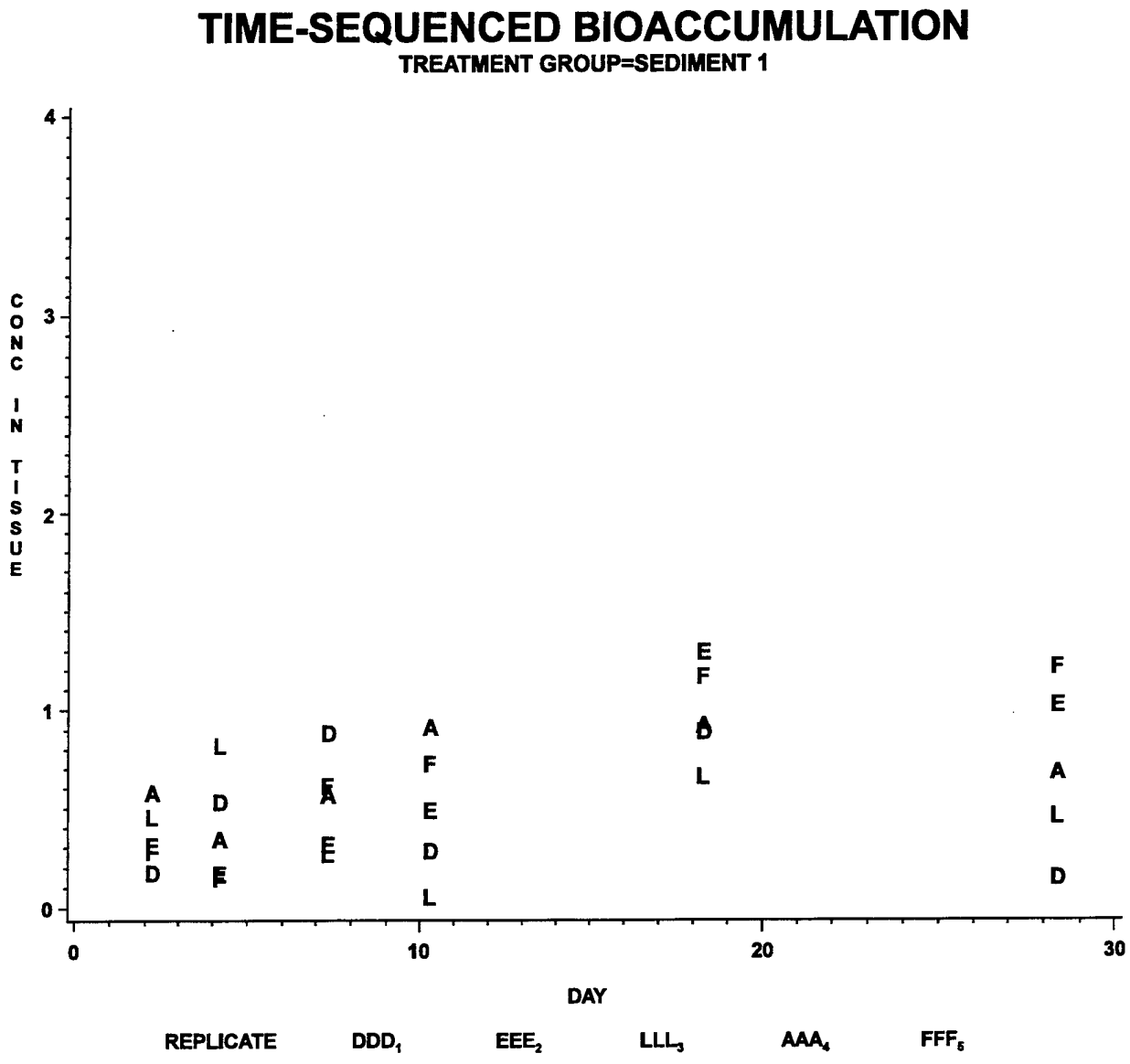


Figure D-9. Plot of Time-Sequenced Bioaccumulation Dredged Sediment 1 Example Data by Replicate.

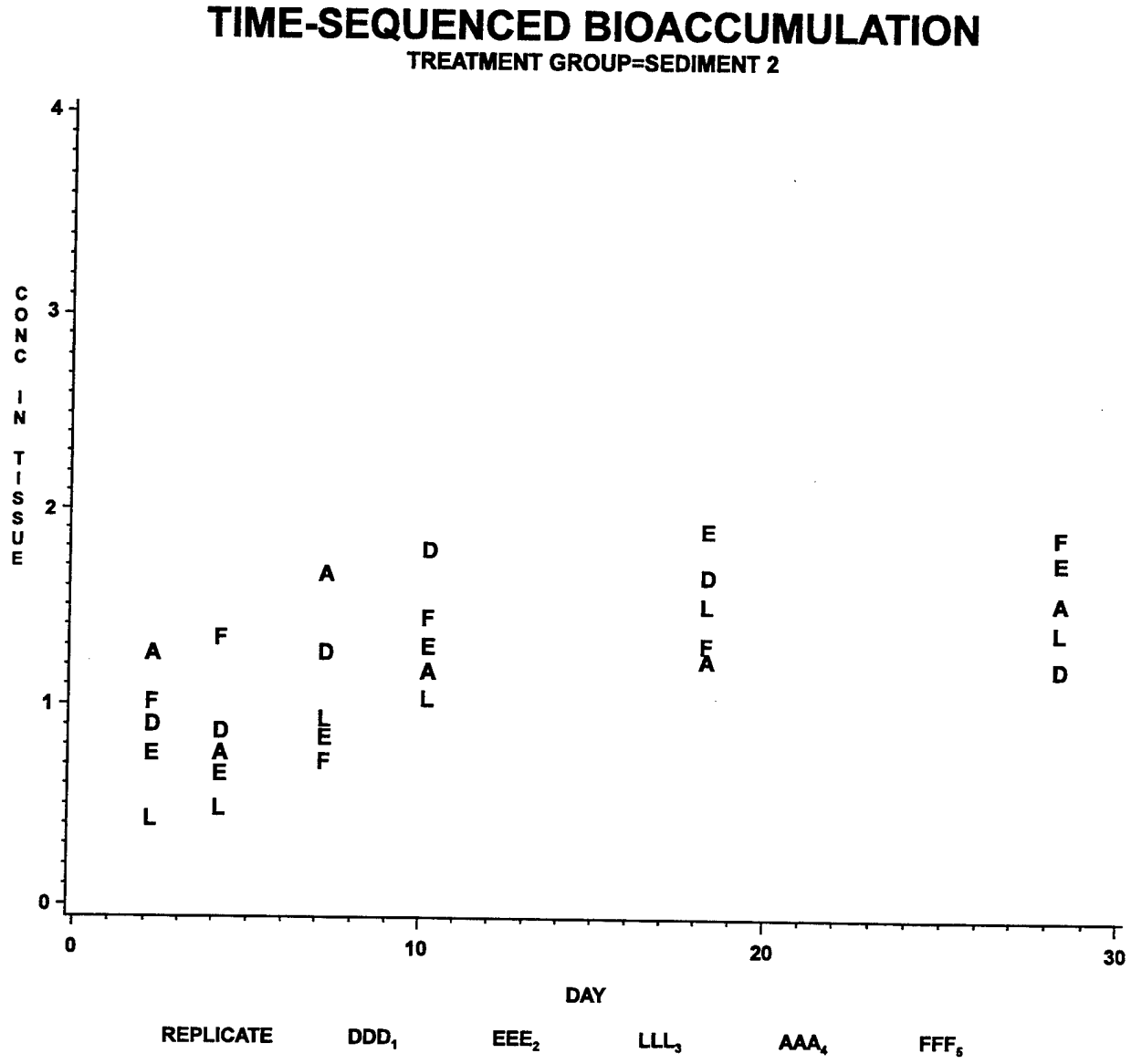


Figure D-10. Plot of Time-Sequenced Bioaccumulation Dredged Sediment 2 Example Data by Replicate.

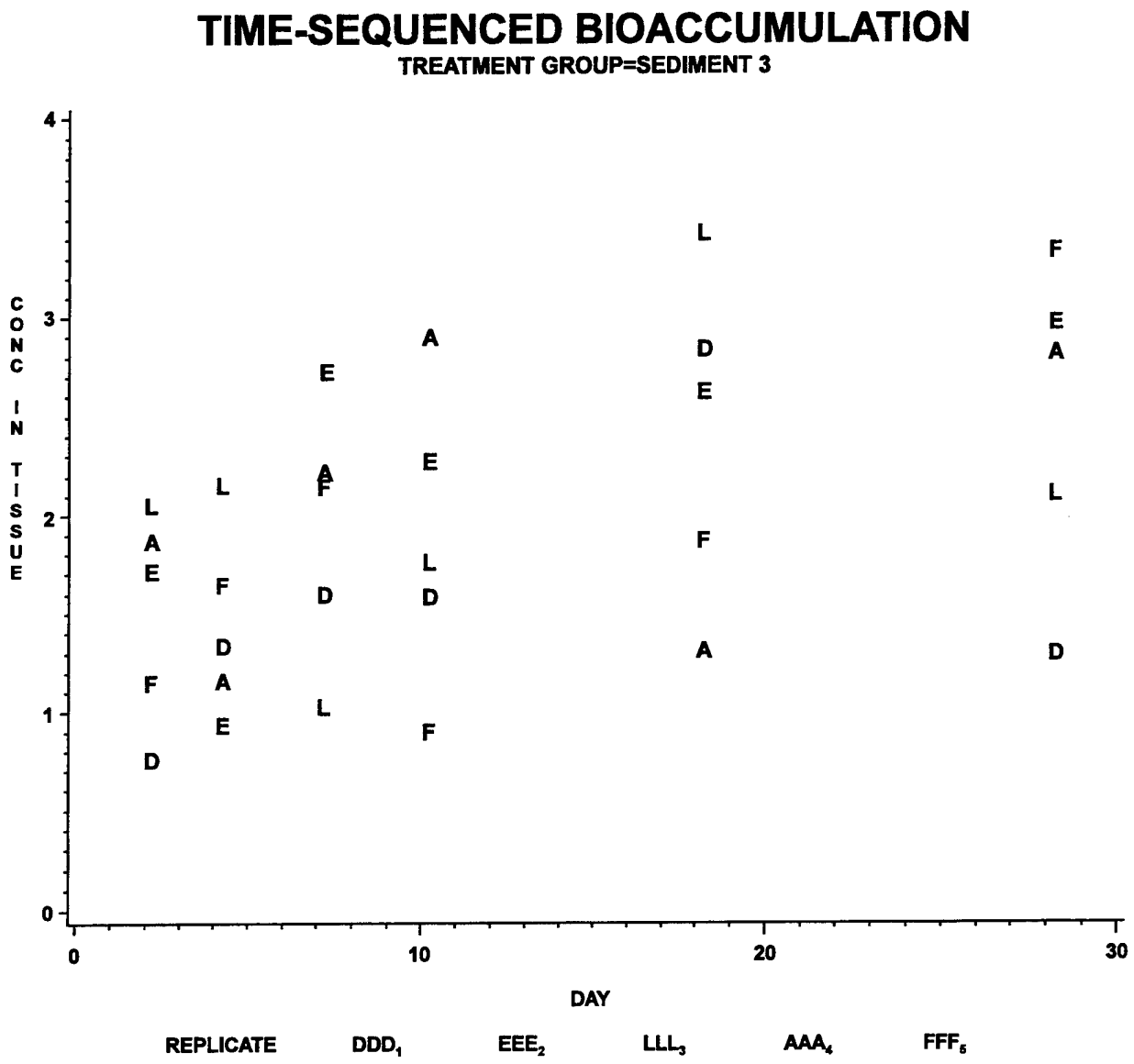


Figure D-11. Plot of Time-Sequenced Bioaccumulation Dredged Sediment 3 Example Data by Replicate.

(Note: the following PROC NLIN output is given as an example only for the reference sediment replicate 1. NLIN output for the other replicates and sediments has been deleted.)

TIME-SEQUENCED BIOACCUMULATION

---------- TREATMENT GROUP=REFERENCE REPLICATE=1 ----------

Non-Linear Least Squares Grid Search Dependent Variable CONC

K1	K2	Sum of Squares
0.300000	0.210000	0.416199
0.400000	0.310000	0.425788
0.500000	0.410000	0.441222
0.200000	0.110000	0.448040
0.400000	0.410000	0.454330
0.600000	0.510000	0.457317
0.300000	0.310000	0.457654
0.500000	0.510000	0.460598
0.600000	0.610000	0.470393
0.700000	0.610000	0.472661

Non-Linear Least Squares DUD Initialization Dependent Variable CONC

DUD	K1	K2	Sum of Squares
-3	0.300000	0.210000	0.416199
-2	0.330000	0.210000	0.461659
-1	0.300000	0.231000	0.405093

Non-Linear Least Squares Iterative Phase Dependent Variable CONC Method: DUD

Iter	K1	K2	Sum of Squares
0	0.300000	0.231000	0.405093
1	0.239451	0.178897	0.400026
2	0.241348	0.179839	0.400014
3	0.241312	0.179738	0.400013
4	0.237752	0.176113	0.399983
5	0.237547	0.175943	0.399983
6	0.237563	0.175943	0.399983
7	0.237360	0.175718	0.399983
8	0.237337	0.175695	0.399983

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable CONC

Source	DF	Sum of Squares	Mean Square
Regression	2	1.2676841229	0.6338420614
Residual	4	0.3999828771	0.0999957193
Uncorrected Total	6	1.6676670000	
(Corrected Total)	5	0.5505135000	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
K1	0.2373370301	0.22487054331	-.38699524147	0.86166930175
K2	0.1756952550	0.21727444929	-.42754716392	0.77893767392

TIME-SEQUENCED BIOACCUMULATION

OBS	TREATMENT GROUP	REPLICATE	UPTAKE RATE CONSTANT, k1	DEPURATION RATE CONSTANT, k2	STEADY STATE CONC., Css	Log10 Css	NORMALIZED RANK FOR Css
1	REFERENCE	1	0.23734	0.17570	0.60788	-0.21618	-0.74414
2	REFERENCE	2	0.30596	0.20060	0.68636	-0.16345	-0.58946
3	REFERENCE	3	0.53975	0.40677	0.59712	-0.22394	-0.91914
4	REFERENCE	4	0.31799	0.16208	0.88285	-0.05411	-0.31457
5	REFERENCE	5	0.04515	0.08670	0.23434	-0.63015	-1.86824
6	SEDIMENT 1	1	0.05916	0.42709	0.55411	-0.25641	-1.12814
7	SEDIMENT 1	2	0.01924	0.04682	1.64392	0.21588	0.44777
8	SEDIMENT 1	3	0.24301	2.20563	0.44071	-0.35584	-1.40341
9	SEDIMENT 1	4	0.05059	0.24290	0.83305	-0.07933	-0.44777
10	SEDIMENT 1	5	0.02419	0.06046	1.60020	0.20418	0.31457
11	SEDIMENT 2	1	0.01439	0.31909	1.48791	0.17258	0.06193
12	SEDIMENT 2	2	0.00653	0.11306	1.90667	0.28028	0.58946
13	SEDIMENT 2	3	0.00548	0.11964	1.51129	0.17935	0.18676
14	SEDIMENT 2	4	0.03430	0.87782	1.28959	0.11045	-0.18676
15	SEDIMENT 2	5	0.02323	0.56773	1.35040	0.13046	-0.06193
16	SEDIMENT 3	1	0.01117	0.25025	1.96371	0.29308	0.74414
17	SEDIMENT 3	2	0.01490	0.23622	2.77595	0.44341	1.86824
18	SEDIMENT 3	3	0.09375	1.97656	2.08697	0.31952	0.91914
19	SEDIMENT 3	4	0.02351	0.45781	2.25943	0.35400	1.12814
20	SEDIMENT 3	5	0.00838	0.13921	2.64810	0.42293	1.40341

TIME-SEQUENCED BIOACCUMULATION

OBS	TREATMENT GROUP	N	MEAN Css	VARIANCE	STANDARD ERROR	MEAN Log10 Css	VARIANCE OF LOGS	STANDARD ERROR OF LOGS
1	REFERENCE	5	0.60171	0.05531	0.10517	-0.25757	0.047978	0.09796
2	SEDIMENT 1	5	1.01440	0.32833	0.25625	-0.05430	0.068052	0.11666
3	SEDIMENT 2	5	1.50917	0.05797	0.10768	0.17462	0.004314	0.02937
4	SEDIMENT 3	5	2.34683	0.12421	0.15761	0.36659	0.004214	0.02903

TIME-SEQUENCED BIOACCUMULATION
SHAPIRO-WILKS TEST FOR NORMALITY

UNIVARIATE PROCEDURE

Variable=RESID

N	20		
W:Normal	0.963283	Prob<W	0.6122

Variable=RESIDLOG

N	20		
W:Normal	0.942525	Prob<W	0.2796

TIME-SEQUENCED BIOACCUMULATION
LEVENE'S TEST
General Linear Models Procedure

Dependent Variable: ABSDEV ABSOLUTE DEVIATIONS FROM Css MEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.37008913	0.12336304	4.74	0.0150
Error	16	0.41648071	0.02603004		
Corrected Total	19	0.78656984			

Dependent Variable: ABSLOG ABSOLUTE DEVIATIONS FROM logCss MEAN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.09646576	0.03215525	3.68	0.0344
Error	16	0.13965602	0.00872850		
Corrected Total	19	0.23612178			

TIME-SEQUENCED BIOACCUMULATION
LSD TEST (UNTRANSFORMED DATA)
General Linear Models Procedure

T tests (LSD) for variable: CSS

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.1 df= 16 MSE= 0.141456
Critical Value of T= 1.75
Least Significant Difference= 0.4153

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	2.347	5	SEDIMENT 3
B	1.509	5	SEDIMENT 2
C	1.014	5	SEDIMENT 1
C			
C	0.602	5	REFERENCE

TIME-SEQUENCED BIOACCUMULATION
LSD TEST (LOG-TRANSFORMED DATA)
General Linear Models Procedure

T tests (LSD) for variable: LOGCSS

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.1 df= 16 MSE= 0.03114
Critical Value of T= 1.75
Least Significant Difference= 0.1949

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	0.367	5	SEDIMENT 3
A			
A	0.175	5	SEDIMENT 2
B	-0.054	5	SEDIMENT 1
C	-0.258	5	REFERENCE

TIME-SEQUENCED BIOACCUMULATION
T-TEST

TTEST PROCEDURE

Variable: CSS STEADY STATE CONC., Css

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	0.60171086	0.23517166	0.10517196
SEDIMENT 1	5	1.01440008	0.57300347	0.25625494

Variances	T	DF	Prob>|T|
Unequal	-1.4899	5.3	0.1935
Equal	-1.4899	8.0	0.1746

For H0: Variances are equal, F' = 5.94 DF = (4,4) Prob>F' = 0.1127

Variable: LOGCSS Log10 Css

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.25756572	0.21903881	0.09795713
SEDIMENT 1	5	-0.05430384	0.26086789	0.11666367

Variances	T	DF	Prob>|T|
Unequal	-1.3343	7.8	0.2200
Equal	-1.3343	8.0	0.2188

For H0: Variances are equal, F' = 1.42 DF = (4,4) Prob>F' = 0.7431

TTEST PROCEDURE

Variable: CSS STEADY STATE CONC., Css

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	0.60171086	0.23517166	0.10517196
SEDIMENT 2	5	1.50916957	0.24077410	0.10767745

Variances	T	DF	Prob>|T|
Unequal	-6.0289	8.0	0.0003
Equal	-6.0289	8.0	0.0003

For H0: Variances are equal, F' = 1.05 DF = (4,4) Prob>F' = 0.9647

Variable: LOGCSS Log10 Css

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.25756572	0.21903881	0.09795713
SEDIMENT 2	5	0.17462207	0.06568351	0.02937456

Variances	T	DF	Prob>|T|
Unequal	-4.2261	4.7	0.0097
Equal	-4.2261	8.0	0.0029

For H0: Variances are equal, F' = 11.12 DF = (4,4) Prob>F' = 0.0386

TTEST PROCEDURE

Variable: CSS STEADY STATE CONC., Css

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	0.60171086	0.23517166	0.10517196
SEDIMENT 3	5	2.34683295	0.35243662	0.15761445

Variances	T	DF	Prob>|T|
Unequal	-9.2100	7.0	0.0001
Equal	-9.2100	8.0	0.0000

For H0: Variances are equal, F' = 2.25 DF = (4,4) Prob>F' = 0.4525

Variable: LOGCSS Log10 Css

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.25756572	0.21903881	0.09795713
SEDIMENT 3	5	0.36658794	0.06491256	0.02902978

Variances	T	DF	Prob>|T|
Unequal	-6.1091	4.7	0.0023
Equal	-6.1091	8.0	0.0003

For H0: Variances are equal, F' = 11.39 DF = (4,4) Prob>F' = 0.0370

TIME-SEQUENCED BIOACCUMULATION
Css CONVERTED TO RANKITS
SHAPIRO-WILKS TEST FOR NORMALITY

UNIVARIATE PROCEDURE

Variable=RESID

N	20		
W:Normal	0.970187	Prob<W	0.7497

TIME-SEQUENCED BIOACCUMULATION
Css CONVERTED TO RANKITS
LEVENE'S TEST

General Linear Models Procedure

Dependent Variable: ABSDEV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.52458729	0.17486243	1.88	0.1741
Error	16	1.49037397	0.09314837		
Corrected Total	19	2.01496126			

TIME-SEQUENCED BIOACCUMULATION
Css CONVERTED TO RANKITS
LSD TEST

General Linear Models Procedure
T tests (LSD) for variable: RANKIT

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.1 df= 16 MSE= 0.33088
Critical Value of T= 1.75
Least Significant Difference= 0.6352

Means with the same letter are not significantly different.

T Grouping	Mean	N	TRT
A	1.213	5	SEDIMENT 3
B	0.118	5	SEDIMENT 2
B			
C B	-0.443	5	SEDIMENT 1
C			
C	-0.887	5	REFERENCE

TIME-SEQUENCED BIOACCUMULATION
Css CONVERTED TO RANKITS
T-TEST

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE CSS

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.88710960	0.59170982	0.26462068
SEDIMENT 1	5	-0.44339680	0.83054481	0.37143093

Variances	T	DF	Prob>|T|
Unequal	-0.9729	7.2	0.3621
Equal	-0.9729	8.0	0.3591

For H0: Variances are equal, F' = 1.97 DF = (4,4) Prob>F' = 0.5275

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE CSS

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.88710960	0.59170982	0.26462068
SEDIMENT 2	5	0.11789116	0.29807434	0.13330290

Variances	T	DF	Prob>|T|
Unequal	-3.3918	5.9	0.0151
Equal	-3.3918	8.0	0.0095

For H0: Variances are equal, F' = 3.94 DF = (4,4) Prob>F' = 0.2126

TTEST PROCEDURE

Variable: RANKIT RANK FOR VARIABLE CSS

TRT	N	Mean	Std Dev	Std Error
REFERENCE	5	-0.88710960	0.59170982	0.26462068
SEDIMENT 3	5	1.21261524	0.44129976	0.19735525

Variances	T	DF	Prob>|T|
Unequal	-6.3607	7.4	0.0003
Equal	-6.3607	8.0	0.0002

For H0: Variances are equal, F' = 1.80 DF = (4,4) Prob>F' = 0.5839

TIME-SEQUENCED BIOACCUMULATION
POWER OF LSD TO DETECT A TRUE POPULATION DIFFERENCE (D)
ABOVE REFERENCE MEAN Css

NO. OF REPLICATES, N	REFERENCE MEAN Css	MEAN SQUARE ERROR, MSE	DEGREES OF FREEDOM, DF	T VALUE FOR (1-ALPHA=0.95,DF)
5	0.60171	0.14146	16	1.74588

POWER OF LSD TO DETECT % INCREASE IN Css ABOVE REFERENCE
MEAN Css GIVEN N, MSE AND DF SHOWN ABOVE

% INCREASE IN Css ABOVE REFERENCE	DREDGED SEDIMENT Css	D	T VALUE FOR (1-BETA,DF)	POWER (1-BETA)
10	0.66188	0.06017	-1.49293	0.07746
25	0.75214	0.15043	-1.11349	0.14097
50	0.90257	0.30086	-0.48110	0.31848
100	1.20342	0.60171	0.78369	0.77767
200	1.80513	1.20342	3.31327	0.99780
300	2.40684	1.80513	5.84285	0.99999

MINIMUM DREDGED SEDIMENT Css THAT CAN BE DETECTED BY LSD
AS SIGNIFICANT GIVEN SPECIFIED POWER AND N, MSE, AND DF SHOWN ABOVE

POWER (1-BETA)	D	DREDGED SEDIMENT Css	% INCREASE IN Css ABOVE REFERENCE	T VALUE FOR (1-BETA,DF)
0.50	0.41529	1.01700	69.019	0.00000
0.60	0.47657	1.07828	79.202	0.25760
0.70	0.54256	1.14427	90.169	0.53501
0.80	0.62097	1.22268	103.201	0.86467
0.90	0.73327	1.33498	121.864	1.33676
0.95	0.83059	1.43230	138.038	1.74588
0.99	1.02983	1.63154	171.150	2.58349

COMPARISON OF MEAN DREDGED SEDIMENT Css WITH ACTION LEVEL:
UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE EQUAL

TREATMENT GROUP	MEAN DREDGED SEDIMENT Css	UCL (EQUAL VARIANCES)	MEAN SQUARE ERROR	T VALUE FOR (1-ALPHA=.95,DF)	DF	MINIMUM SIGNIFICANT DIFFERENCE
SEDIMENT 1	1.01440	1.30806	0.14146	1.74588	16	0.29366
SEDIMENT 2	1.50917	1.80283	0.14146	1.74588	16	0.29366
SEDIMENT 3	2.34683	2.64049	0.14146	1.74588	16	0.29366

COMPARISON OF MEAN DREDGED SEDIMENT Css WITH ACTION LEVEL:
UPPER CONFIDENCE LIMITS (UCL) WHEN VARIANCES ARE UNEQUAL

TREATMENT GROUP	MEAN DREDGED SEDIMENT Css	UCL (UNEQUAL VARIANCES)	VARIANCE	T VALUE FOR (1-ALPHA=.95,N-1)	N	MINIMUM SIGNIFICANT DIFFERENCE
SEDIMENT 1	1.01440	1.56070	0.32833	2.13185	5	0.54630
SEDIMENT 2	1.50917	1.73872	0.05797	2.13185	5	0.22955
SEDIMENT 3	2.34683	2.68284	0.12421	2.13185	5	0.33601

POWER TO DETECT % DECREASE IN Css BELOW
ACTION LEVEL OF 2 ug/g GIVEN N, MSE AND DF SHOWN ABOVE

% DECREASE BELOW ACTION LEVEL	DREDGED SEDIMENT Css	D	T VALUE FOR (1-BETA,DF)	POWER (1-BETA)
10	1.8	0.2	-0.55682	0.29268
20	1.6	0.4	0.63224	0.73192
30	1.4	0.6	1.82131	0.95634
40	1.2	0.8	3.01037	0.99585
50	1.0	1.0	4.19943	0.99966

D4.5 SAS Program Statements for Censored Data Methods

SAS statements are given for the censored data methods DL, DL/2, ZERO, UNIF, and LR. Appropriate censored data methods from Table D-12 should be applied to bioaccumulation data sets that contain nondetects, prior to running BIOACC.SAS or BIOACCSS.SAS. The revised concentration data set obtained from the selected censored data method may then be used as the input data set for BIOACC.SAS or BIOACCSS.SAS.

First, create a contaminant concentration data set as in BIOACC.SAS (note that some of the concentrations have been changed from BIOACC.SAS in order to illustrate the censored data methods):

```
LIBNAME Q 'C:\SAS';
DATA BIOACC;
 INPUT TRT REP CONC @@;
 CARDS;
1 1 -.06 1 2 -.06 1 3 -.06 1 4 -.06 1 5 .09
2 1 -.06 2 2  .19 2 3  .18 2 4  .33 2 5 .31
3 1  .24 3 2  .10 3 3  .13 3 4  .18 3 5 .30
4 1  .13 4 2 -.06 4 3  .17 4 4 -.06 4 5 2.2
;
```

The minus signs are a convenient way of indicating nondetects and do not imply negative concentrations. All SAS programs that follow assume that nondetects have been coded as negatives. In the data above, DL = 0.06. This example data set is 35% censored, has unequal variances that increase as the means increase, CV equal to 2.0, and is lognormally or nonnormally distributed. The variance and distribution characteristics were determined by applying several of the methods that follow, and then testing the data for equality of variances using Levene's Test, and for normality and lognormality of residuals using Shapiro-Wilk's Test. From Table D-12, one would select and apply either DL or DL/2, and then proceed with BIOACC.SAS, using log-transformed data or rankits as appropriate. If rankits are needed, the method UNIF could also be used.

D4.5.1 SAS Statements for DL, DL/2 and ZERO

Read the data set created above into a new set, assign the DL, and use the statement corresponding to the selected simple substitution method:

```
DATA Q.BIOACC;
 SET BIOACC;
 IF CONC<0 THEN DL=ABS(CONC);
 OCONC=CONC;
 IF CONC<0 THEN CONC=DL;       /* Include this statement if using DL */
 IF CONC<0 THEN CONC=DL/2;     /* Include this statement if using DL/2 */
 IF CONC<0 THEN CONC=0; /* Include this statement if using ZERO */
PROC PRINT LABEL;              /* Print the revised data set */
 VAR TRT REP OCONC CONC DL;
 LABEL TRT='TREATMENT GROUP'
       REP='REPLICATE'
       OCONC='ORIGINAL CONCENTRATION'
       CONC='REVISED CONCENTRATION'
       DL='DETECTION LIMIT';
 TITLE 'Uncensoring Using Simple Substitution Methods';
```

D4.5.1.1 SAS Program Output for DL, DL/2, or ZERO

Uncensoring Using Simple Substitution Methods

OBS	TREATMENT GROUP	REPLICATE	ORIGINAL CONCEN-TRATION	REVISED CONCEN-TRATION (DL)	REVISED CONCEN-TRATION (DL/2)	REVISED CONCEN-TRATION (ZERO)	DETECTION LIMIT
1	1	1	-0.06	0.06	0.03	0.00	0.06
2	1	2	-0.06	0.06	0.03	0.00	0.06
3	1	3	-0.06	0.06	0.03	0.00	0.06
4	1	4	-0.06	0.06	0.03	0.00	0.06
5	1	5	0.09	0.09	0.09	0.09	.
6	2	1	-0.06	0.06	0.03	0.00	0.06
7	2	2	0.19	0.19	0.19	0.19	.
8	2	3	0.18	0.18	0.18	0.18	.
9	2	4	0.33	0.33	0.33	0.33	.
10	2	5	0.31	0.31	0.31	0.31	.
11	3	1	0.24	0.24	0.24	0.24	.
12	3	2	0.10	0.10	0.10	0.10	.
13	3	3	0.13	0.13	0.13	0.13	.
14	3	4	0.18	0.18	0.18	0.18	.
15	3	5	0.30	0.30	0.30	0.30	.
16	4	1	0.13	0.13	0.13	0.13	.
17	4	2	-0.06	0.06	0.03	0.00	0.06
18	4	3	0.17	0.17	0.17	0.17	.
19	4	4	-0.06	0:06	0.03	0.00	0.06
20	4	5	2.20	2.20	2.20	2.20	.

D4.5.2 SAS Statements for UNIF

Create a contaminant concentration data set as in the first step above. Now, define DL and count number of reps (NREP) and censored (NC) and uncensored observations (NUC) in each treatment.

```
DATA A;
 SET BIOACC;
 IF CONC<0 THEN DL=ABS(CONC);
 OCONC=CONC;
 IF CONC<0 THEN COUNT=1; ELSE COUNT=0;
PROC MEANS NOPRINT;
 BY TRT;
 VAR COUNT;
 OUTPUT OUT=B0 SUM=NC N=NREP;
DATA B; SET B0;
 NUC=NREP-NC;
 DROP _TYPE_ _FREQ_;

/* The following statements inititalize a counter at the first observation of each
treatment, and then implement the UNIF formula.  If there is only one nondetect in a
treatment, it is set equal to DL/2. */

DATA Q.BIOACC;
 MERGE A B; BY TRT;
 IF FIRST.TRT THEN I=1;
 IF CONC<0 THEN DO;
  CONC=DL*(I-1)/(NC-1);
  IF NC=1 THEN CONC=DL/2;
  I+1;
  END;
PROC PRINT LABEL;              /* Print the revised data set */
 VAR TRT REP OCONC CONC DL;
 LABEL TRT='TREATMENT GROUP'
       REP='REPLICATE'
       OCONC='ORIGINAL CONCENTRATION'
       CONC='REVISED CONCENTRATION'
       DL='DETECTION LIMIT';
 TITLE 'Uncensoring Using UNIF';
```

D4.5.2.1 SAS Program Output for UNIF

Uncensoring Using UNIF

OBS	TREATMENT GROUP	REPLICATE	ORIGINAL CONCENTRATION	REVISED CONCENTRATION	DETECTION LIMIT
1	1	1	-0.06	0.00	0.06
2	1	2	-0.06	0.02	0.06
3	1	3	-0.06	0.04	0.06
4	1	4	-0.06	0.06	0.06
5	1	5	0.09	0.09	.
6	2	1	-0.06	0.03	0.06
7	2	2	0.19	0.19	.
8	2	3	0.18	0.18	.
9	2	4	0.33	0.33	.
10	2	5	0.31	0.31	.
11	3	1	0.24	0.24	.
12	3	2	0.10	0.10	.
13	3	3	0.13	0.13	.
14	3	4	0.18	0.18	.
15	3	5	0.30	0.30	.
16	4	1	0.13	0.13	.
17	4	2	-0.06	0.00	0.06
18	4	3	0.17	0.17	.
19	4	4	-0.06	0.06	0.06
20	4	5	2.20	2.20	.

D4.5.3 SAS Statements for LR

Create a contaminant concentration data set as in the methods above. Now, define DL and count number of reps (NREP) and censored (NC) and uncensored observations (NUC) in each treatment, same as for UNIF above.

```
DATA A;
 SET BIOACC;
 IF CONC<0 THEN DL=ABS(CONC);
 OCONC=CONC;
 IF CONC<0 THEN COUNT=1; ELSE COUNT=0;
PROC MEANS NOPRINT;
 BY TRT;
 VAR COUNT;
 OUTPUT OUT=B0 SUM=NC N=NREP;
DATA B; SET B0;
 NUC=NREP-NC;
 DROP _TYPE_ _FREQ_;

/* LR should not be used unless there are at least 3 uncensored observations in a
treatment. If a treatment has more than one nondetect, each nondetect must be assigned
a different value below the DL. When nondetects have been originally scored as
negative concentrations, this can be done easily by multiplying each negative concen-
tration by its rep number. */

DATA C;
 MERGE A B; BY TRT;
 IF NUC<3 THEN DELETE;
 IF CONC<0 THEN CONC=CONC*REP;

/* Assign normal scores (rankits) to all concentrations and store in variable RANKIT
*/

PROC RANK NORMAL=BLOM OUT=C1;
 BY TRT; VAR CONC; RANKS RANKIT;

/* Make a new data set including only above-DL observations. These will be used with
their rankits in the REG procedure to calculate regression parameters. */

DATA C2; SET C1;
 IF CONC<0 THEN DELETE;
 SLOPE=RANKIT;
 LOGCONC=LOG10(CONC);                /* Take logs of above-DL concentrations */

/* Regress logs of above-DL concentrations against their rankits and output the
regression parameters */

PROC REG NOPRINT OUTEST=D;
 BY TRT;
 MODEL LOGCONC=SLOPE;
```

```
/* Make a new data set of just the nondetects. Merge it with the set of regression
parameters. Then estimate log concentrations for the nondetects using the slope and
intercept from the regression model, and the previously calculated rankits of the
nondetects. Take the antilogs to obtain estimated concentrations for the nondetects.
One problem with the LR method is that regression estimates of concentrations for
nondetects may exceed the DL. In such cases the concentration should be set equal to
the DL. */

DATA C3; SET C1;
 IF CONC<0;
DATA D1;
 MERGE D C3; BY TRT;
 LOGCONC=INTERCEP+SLOPE*RANKIT;
 CONC=10**LOGCONC;
 IF CONC=. THEN DELETE;
 IF CONC>DL THEN CONC=DL;

/* Combine with above-DL observations. Sort the data and print. Note that the new
data set will not include any treatments having fewer than 3 above-DL observations. */

DATA Q.BIOACC;
 SET C2 D1;
PROC SORT; BY TRT REP;
PROC PRINT LABEL;
 VAR TRT REP OCONC LRCONC CONC DL;
 LABEL TRT='TREATMENT GROUP'
       REP='REPLICATE'
       OCONC='ORIGINAL CONCENTRATION'
       LRCONC='CONCENTRATION ESTIMATED BY LR'
       CONC='REVISED CONCENTRATION'
       DL='DETECTION LIMIT';
 TITLE 'Uncensoring with LR';
```

D4.5.3.1 SAS Program Output for LR

Uncensoring with LR

OBS	TREATMENT GROUP	REPLICATE	ORIGINAL CONCENTRATION	CONCENTRATION ESTIMATED BY LR	REVISED CONCENTRATION	DETECTION LIMIT
1	2	1	-0.06	0.13291	0.06000	0.06
2	2	2	0.19	.	0.19000	.
3	2	3	0.18	.	0.18000	.
4	2	4	0.33	.	0.33000	.
5	2	5	0.31	.	0.31000	.
6	3	1	0.24	.	0.24000	.
7	3	2	0.10	.	0.10000	.
8	3	3	0.13	.	0.13000	.
9	3	4	0.18	.	0.18000	.
10	3	5	0.30	.	0.30000	.
11	4	1	0.13	.	0.13000	.
12	4	2	-0.06	0.02662	0.02662	0.06
13	4	3	0.17	.	0.17000	.
14	4	4	-0.06	0.00490	0.00490	0.06
15	4	5	2.20	.	2.20000	.

D5.0 REFERENCES

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APPENDIX E
SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR TIER III BIOASSAYS

Acute Toxicity
Water Column Tests

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSID SHRIMP, *Mysidopsis bahia, M. bigelowi, M. almyra, Neomysis americana, Holmesimysis costata*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	20±1°C: or 25±1°C for *Mysidopsis bahia* *Mysidopsis bigelowi* *Mysidopsis almyra* 20±1°C for *Neomysis americana* 12±1°C for *Holmesimysis costata*
4. Salinity:	25-30 ‰ ±10% except for *Holmesimysis costata* which is to be 32-34 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	250 mL minimum
9. Test solution volume:	200 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	1 - 5 d; 24 h range in age
12. No. organisms per test chamber:	10 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	50 minimum
15. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; feed 0.2 mL of concentrated suspension of *Artemia* nauplii ≤24 h old, daily (approximately 100 nauplii per mysid)
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 40% for: *Mysidopsis bahia* *Mysidopsis bigelowi* *Mysidopsis almyra* *Neomysis americana* and DO> 60% saturation for: *Holmesimysis costata* (< 100 bubbles/min.)
18. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival

22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	1 L per site
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR GRASS SHRIMP, *Palaemonetes* sp., ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	25±1°C
4. Salinity:	30-35 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	1 L minimum
9. Test solution volume:	750 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	1-4 d from hatch
12. No. organisms per test chamber:	10 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	50 minimum
15. Feeding regime:	None
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
18. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	4 L per site minimum
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

Modified from the mysid acute toxicity water column test published in:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR COMMERCIAL SHRIMP, *Penaeus* sp., ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	25±1°C
4. Salinity:	30-35 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	80 L
9. Test solution volume:	60 L
10. Renewal of test solutions:	None
11. Age of test organisms:	8-10 d post larvae
12. No. organisms per test chamber:	10 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	50 minimum
15. Feeding regime:	None
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
18. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	20 L for site sediment
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

Modified from the mysid shrimp acute toxicity water column test published in:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE CLADOCERANS, *Daphnia magna* AND *D. pulex,* ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	20 or 25±1°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	30 mL minimum
9. Test solution volume:	25 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	Less than 24 h old
12. No. organisms per test chamber:	5 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	25 minimum
15. Feeding regime:	Feed YCT* and *Selenastrum* while holding prior to the test; newly-released young should have food available a minimum of 2 h prior to use in a test; add 0.2 mL each of YCT and *Selenastrum* at -2 h and at 48 h.
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	1 L per site
24. Test acceptability criterion:	≥ 90% survival in controls

* Slurry of Yeast, Cereal flakes, Trout chow.

REFERENCE:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE CLADOCERAN, *Ceriodaphnia dubia,* ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	20 or 25±1°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20- uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	30 mL minimum
9. Test solution volume:	15 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	Less than 24 h old
12. No. organisms per test chamber:	5 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	25 minimum
15. Feeding regime:	Feed YCT* and *Selenastrum* while holding prior to the test: newly-released young should have food available a minimum of 2 h prior to use in a test: add 0.1 mL each of YCT and *Selenastrum* at -2 h and at 48 h
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent or deionized water and reagent grade chemicals, or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	1 L per site
24. Test acceptability criterion:	≥ 90% survival in controls

* Slurry of Yeast, Cereal flakes, Trout chow.

REFERENCE:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SHEEPSHEAD MINNOW, *Cyprinodon variegatus*, INLAND SILVERSIDE, *Menidia beryllina*, ATLANTIC SILVERSIDE, *M. menidia*, TIDEWATER SILVERSIDE, *M. peninsulae*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	20 or 25±1°C
4. Salinity:	Sheepshead minnow: 5-30 ‰ ± 10% Silversides: 5-32 ‰ ± 10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	250 mL minimum
9. Test solution volume:	200 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	Sheepshead minnow: 1 - 14 d; 24-h range in age Silversides: 9 - 14 d; 24-h range in age
12. No. organisms per test chamber:	10 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	50 minimum
15. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; add 0.2 mL *Artemia* nauplii concentrate at 48 h
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
18. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millpore MILLI-Q® or equivalent or deionized water
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	4 L per site
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE SPECKLED SANDDAB, *Citharichthys stigmaeus*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test Duration:	96 h
3. Temperature:	15±2°C
4. Salinity:	30±2 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 μE/m²/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	30 L
9. Test solution volume:	20 L
10. Renewal of test organisms:	None
11. Age of test organisms:	Juveniles ≤ 8 cm
12. No. organisms per test chamber:	10
13. No. replicate chambers per concentration:	5 minimum
14 No. organisms per concentration:	50 minimum
15. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test: add 0.2 mL *Artemia* nauplii concentrate at 48 h
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
18. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	20 L for site sediment
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

Adapted in part from the *Menidia* sp. protocol published in:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90-027.

and from EPA in-house expertise, ERL-Narragansett, RI.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR GRUNION, *Leuresthes tenuis*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	20 or 25±2°C
4. Salinity:	20-32 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	250 mL minimum
9. Test solution volume:	200 mL minimum
10. Renewal of test organisms:	None
11. Age of test organisms:	9 - 14 d
12. No. organisms per test chamber:	10
13. No. of replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	50 minimum
15. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test: add 0.2 mL *Artemia* nauplii concentrate at 48 h
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 40% saturation (<100 bubbles/min.)
18. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artifical seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirments:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	20 L for site sediment
24. Test acceptability criterion:	≥ 90% or greater survival in controls

REFERENCE:

Adapted in part from the *Menidia* sp. protocol published in:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027

and from personal communications with Dr. Doug Middaugh, EPA, ERL-Gulf Breeze, FL.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR FATHEAD MINNOW, *Pimephales promelas*, BLUEGILL SUNFISH, *Lepomis macrochirus*, AND CHANNEL CATFISH, *Ictalurus punctatus*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	20 or 25±1°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	250 mL minimum
9. Test solution volume:	200 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	Fathead minnow - on order of 4 d; 24 h range in age. Sunfish and Catfish - on order of 30 d
12. No. organisms per test chamber:	10 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	50 minimum
15. Feeding regime:	*Artemia* nauplii are made available while holding prior to the test; add 0.2 mL *Artemia* nauplii concentrate at 48 h
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
18. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	4L per site minimum
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR RAINBOW TROUT, *Oncorhynchus mykiss*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	12±1°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m²/s (50-100 ft-c)
7. Photoperiod:	16L/8D: Light intensity should be raised gradually over a 15 min period at the beginning of the photoperiod, and lowered gradually at the end of the photoperiod, using a dimmer switch or other suitable device
8. Test chamber size:	5 L minimum, test chambers should be covered to prevent fish from jumping out
9. Test solution volume:	4 L minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	15-30 d (after yolk sac absorption to 30 d)
12. No. organisms per test chamber:	10 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	50 minimum
15. Feeding regime:	Feeding not required
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 60% saturation (< 100 bubbles/min.)
18. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	20 L for site sediment
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR OYSTER, *Crassostrea virginica,* AND MUSSEL, *Mytilus edulis*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	48 h
3. Temperature:	25±1° C for *Crassostrea virginica* 16±1° C for *Mytilus edulis*
4. Salinity:	18-32± 1 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:*	1 L
9. Test solution volume:*	500 mL
10. Renewal of test solutions:	None
11. Age of test organisms:	Larvae less than 4 h old
12. No. organisms per test chamber:	7,500 - 15,000
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	22,500 - 45,000
15. Feeding regime:	None
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. Dilution water:*	Natural seawater or modified GP2, Forty Fathoms®, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	None
21. Endpoint:	Shell development to hinged, D-shaped prodissoconch I larva
22. Sampling and sample	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	1 L per site
24. Test acceptability * criterion:	≥ 70% or greater survival and ≥ 70% shell development in controls

* - Protocol dependent

REFERENCE:

ASTM. 1989. E 724-89. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SEA URCHINS, *Strongylocentrotus* sp., *Lytechinus pictus,* AND SAND DOLLAR, *Dendraster* sp., ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	48 h
3. Temperature:	12°C
4. Salinity:	30-32 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Not essential
8. Test chamber size:	20 mL minimum
9. Test solution volume:	10 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	≤ 1 h embryos
12. No. organisms per test chamber:	2000
13. No. replicate chambers per concentration:	3 minimum
14. No. organisms per concentration:	6000 minimum
15. Feeding regime:	None
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water and 3x brine to maintain constant salinity across tests
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50%, 10%
21. Endpoint:	Survival, Embryo Development
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	1 L per site
24. Test acceptability criterion:	≥ 70% survival and ≥ 70% normal embryo development in controls

REFERENCE:

USEPA. 1990. Conducting the Sea Urchin Larval Development Test. ERL-Narragansett Standard Operating Procedure 1.03.007.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SEA URCHIN, *Strongylocentrotus purpuratus,* AND SAND DOLLAR, *Dendraster excentricus*, SPERM CELL ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	80 minute (60 minute exposure plus 20 minute fertilization period)
3. Temperature:	12°C
4. Salinity:	30±2 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Not essential
8. Test chamber size:	Test tubes 16 x 100 or 125 mm
9. Test solution volume:	5 mL
10. Renewal of test solutions:	None
11. Age of test organisms:	Fresh eggs and sperm
12. No. organisms per test chamber:	560,000 sperm/1,120 eggs (100 eggs observed)
13. No. replicate chambers per concentration:	3 minimum
14. No. organisms per concentration:	300 eggs observed per concentration
15. Feeding regime:	None
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. Dilution water:	Filtered (0.45 µm): natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water and 3x brine to maintain constant salinity across tests.
19. Test concentrations:	Three concentrations for site sediment, and control water
20. Dilution series:	100%, 50% 10%
21. Endpoint:	Egg fertilization percentage
22. Sampling and sample holding requirements:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	1 L per site
24. Test acceptability criterion:	≥ 50% control fertilization, sperm:egg ratio between 250:1 and 1,000:1

REFERENCE:

Dinnel, P.A., Q.J. Stober, S.C. Crumley and R.E. Nakatani. 1982. Development of a sperm cell toxicity test for marine waters. Pp. 82-98 In: *Aquatic Toxicity and Hazard Assessment. Fifth Conference.* J.G. Pearson, R.B. Foster, and W.E. Bishop (Eds.). ASTM STP 766. American Society for Testing and Materials, Philadelphia, PA.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Ampelisca abdita*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20°C
4. Salinity:	20 to 35 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Continuous Light
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 950 mL
10. Sediment depth:	4 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Immature amphipods, or mature females only
13. No. of organisms per test chamber:	20 to 30
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100 to 150
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Leptocheirus plumulosus*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20-25°C
4. Salinity:	20 ‰ (range 2 - 32 ‰)
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	1 L
9. Test solution volume	Vol. to 950 mL
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Mature 3 - 5 mm mixed sexes
13. No. of organisms per test chamber:	20
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	N/A
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Schlekat, C.E., B.E. McGee and E. Reinharz. 1992. Testing sediment toxicity in Chesapeake Bay using the amphipod *Leptocheirus plumulosus*: an evaluation. Environ. Toxicol. Chem. 11: 225-236.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Rhepoxynius abronius*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	15 ±3°C
4. Salinity:	28 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Continuous Light
8. Test chamber size:	1 L
9. Test solution volume	Vol. to 950 mL
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Mature 3 - 5 mm mixed sexes
13. No. of organisms per test chamber:	20
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	N/A
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Grandidierella japonica*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	15 - 19 ±3°C
4. Salinity:	30 to 35 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Continuous Light
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 950 mL
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Immature amphipods 3 - 6 mm, no females carrying embryos
13. No. of organisms per test chamber:	20
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100
16. Feeding regime:	Suspension of finely ground Tetramin and the alga *Enteromorpha*
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Corophium* sp., ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	15-25°C
4. Salinity:	Variable, species dependent
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Continuous Light
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 950 mL
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Mature 5 - 8 mm amphipods, mixed sexes
13. No. of organisms per test chamber:	20
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

Adapted from:
ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Eohaustorius estuarius*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	15±3°C
4. Salinity:	2 to ≤28 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Continuous Light
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 950 mL
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Mature amphipods, 3 -5 mm, mixed sexes
13. No. of organisms per test chamber:	20
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE MAYFLY, *Hexagenia limbata*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	17°C, 20-22°C
4. Salinity:	freshwater
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 800 mL
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	young nymphs
13. No. organisms per test chamber:	5 minimum
14. No. replicate chambers per concentrations:	4 minimum
15. No. organisms per concentration:	1-10
16. Feeding regime:	Variable
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	None
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability:	≥ 80% survival in controls

* - Protocol Dependent

REFERENCES:

ASTM. 1994. Method E1383-94. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. In: Annual Book of ASTM Standards, Volume 11.04. American Society for Testing and Materials, Philadelphia, PA.

Bedard, D., A. Hayton and D. Persaud. 1992. Ontario Ministry of the Environment laboratory sediment biological testing protocol. Ontario Ministry of the Environment, Toronto, Ontario. 26 pp.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FRESHWATER AMPHIPOD, *Hyalella azteca*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20 - 25°C
4. Salinity	0-15 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	300 mL minimum
9. Test solution volume:	Variable, depending on test type
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	7 - 14 d
13. No. organisms per test chamber:	10 minimum
14. No. replicate chambers per sediment:	5 minimum
15. No. organisms per sediment:	50 minimum
16. Feeding regime:	Variable (None, Tetrafin, YCT*, rabbit chow, maple leaves)
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (<100 bubbles/min.)
19. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 80% survival in controls

* Slurry of Yeast, Cereal flakes, Trout chow

REFERENCES:

ASTM. 1994. Method E1383-94. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

USEPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-94/024. U.S. Environmental Protection Agency, Duluth, MN.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE POLYCHAETE, *Neanthes arenaceodentata,* ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20 ±1°C
4. Salinity:	20-35 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	12L/12D
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 800 mL
10. Sediment depth:	2.5 cm (200 mL)
11. Renewal of test solutions:	None*
12. Age of test organisms:	2-3 weeks
13. No. organisms per test chamber:	5 maximum
14. No. replicate chambers per concentration:	3-5
15. No. organisms per concentration:	15-25
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms®, or equivalent, artificial seawater prepared with Millipore MILLI-Q® or eqivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

ASTM. 1994. Method E1611-94. Standard guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Dillon, T.M., D.W. Moore and A.B. Gibson. 1993. Development of a chronic sublethal bioassay for evaluating contaminated sediment with the marine polychaete worm, *Nereis (Neanthes) arenaceodentata.* Environ. Toxicol. Chem. 12:589-605.

Reish, D.J. 1992. Guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. ASTM Draft No. 5. July 3, 1992. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE PAPER PONDSHELL FRESHWATER MUSSEL, *Anodonta imbecillis*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	24±1°C
4. Salinity:	0 ‰
5. Light quality:	N/A
6. Light intensity:	N/A
7. Photoperiod:	24 h Dark
8. Test chamber size:	5 cm-diam. glass cylinder closed on lower end with 100 µm Nitex, placed in 250 mL glass dish containing test sediment and overlying water
9. Test solution volume:	150 mL overlying water
10. Sediment depth:	0.5 cm (20 mL)
11. Renewal of test solutions:	None*
12. Age of test organisms:	8-10 d post transformation to juveniles
13. No. organisms per test chamber:	10
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	50 minimum
16. Feeding regime:	Daily; bloomed phytoplankton concentrate @6 mL/L
17. Test chamber cleaning:	None
18. Test solution aeration:	None
19. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW, receiving water or filtered non-toxic natural freshwater
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival (death assumed if absence of ciliary action or empty shells)
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 80% survival in controls

REFERENCES:

Keller, A.K., and S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Andonata imbecilis*. Environ. Toxicol. Chem. 10:539-546.

Warren, L.W. and S.J. Klaine. 1995. The development of freshwater mussel bioassays to characterize sediment toxicity. N. Am. Benthol. Soc. (In Press).

Tennessee Valley Authority Draft Standard Operating Procedures, SOP-21, and personal communication from Don Wade, Tennessee Valley Authority.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSID SHRIMP, *Mysidopsis bahia, M. bigelowi, M. almyra, Neomysis americana, Holmesimysis costata*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20±1°C: or 25±1°C for *Mysidopsis bahia* *Mysidopsis bigelowi* *Mysidopsis almyra* 20±1°C for *Neomysis americana* 12±1°C for *Holmesimysis costata*
4. Salinity:	25-30 ‰ ±10% except for *Holmesimysis costata* which is to be 32-34 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	250 mL (minimum)
9. Test solution volume:	200 mL (minimum)
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	1 - 5 d; 24 h range in age
13. No. organisms per test chamber:	10 minimum
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	50 minimum
16. Feeding regime:	*Artemia* nauplii are made available while holding prior to, but not during, the test; feed 0.2 mL of concentrated suspension of *Artemia* nauplii ≤24 h old, daily (approximately 100 nauplii per mysid)
17. Test chamber cleaning:	None
18. Test solution aeration:	If needed to maintain DO> 40% saturation for: *Mysidopsis bahia* *Mysidopsis bigelowi* *Mysidopsis almyra* *Neomysis americana* and DO> 60% saturation for: *Holmesimysis costata* (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A

22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	1 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

Modified from:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR COMMERCIAL SHRIMP, *Penaeus* sp., ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	25±1°C
4. Salinity:	30-35 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	80 L minimum
9. Test solution volume:	60 L minimum; overlying water variable depending on test type
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	8-10 d post larvae
13. No. organisms per test chamber:	10 minimum
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	50 minimum
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artifical seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	20 L for site sediment and 8 L for reference and control sediment
25. Test acceptability criterion:	≥ 80% survival in controls

REFERENCE:

Modified from the mysid acute toxicity water column test published in:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR GRASS SHRIMP, *Palaemonetes* sp., ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	25±1°C
4. Salinity:	2 ‰ to ≤28 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	80 L minimum
9. Test solution volume:	60 L minimum; overlying water variable depending on test type
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	1-4 d from hatch
13. No. organisms per test chamber:	10 minimum
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	50 minimum
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	If needed to maintain DO > 40% saturation (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	20 L for site sediment and 8 L for reference and control sediment
25. Test acceptability criterion:	≥ 80% survival in controls

REFERENCE:

Modified from the mysid acute toxicity water column test published in:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MIDGES, *Chironomus tentans* AND *C. riparius*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20 or 25°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	300 mL minimum
9. Test solution volume:	100 mL sediment minimum; overlying water variable depending on test type
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None
12. Age of test organisms:	1st - 3rd Instar
13. No. organisms per test chamber:	10 minimum
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	50 minimum
16. Feeding regime:	Variable (None, Tetramin, YCT[t])
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Variable
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	4 L
25. Test acceptability criterion:	≥ 70% survival in controls

[t] Slurry of Yeast, YCT, Trout chow.

REFERENCES:

ASTM. 1994. Method E1383-94. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

USEPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-94/024. U.S. Environmental Protection Agency, Duluth, MN.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE NAIDID OLIGOCHAETE, *Pristina leidyi*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	24±1°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L:8D
8. Test chamber size:	250 mL
9. Test solution volume:	10 g (wet wt)/50 mL overlying water
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Mixed age
13. No. of organisms per test chamber:	5
14. No. replicate chambers per concentration:	5
15. No. organisms per concentration:	25
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	None
19. Dilution water:	Variable
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	500 mL
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

Smith, D.P., J.H. Kennedy and K.L. Dickson. 1991. An evaluation of a naidid oligochaete as a toxicity test organism. Environ. Toxicol. Chem. 10: 1459-1465.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE OLIGOCHAETE, *Tubifex tubifex*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20 - 25°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	250 mL
9. Test solution volume:	100 mL
10. Sediment depth:	100 mL
11 Renewal of test solutions:	None*
12. Age of test organisms:	Mixed age
13. No. organisms per test chamber:	5
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	25
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	None
19. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent, deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	1 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

Adapted from:

ASTM. 1994. Method E1383-94. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Reynoldson, T.B., S.P. Thompson and J.L. Bamsey. 1991. A sediment bioassay using the tubified oligochaete worm *Tubifex tubifex*. Environ. Toxicol. Chem. 10:1061-1072.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE OLIGOCHAETE, *Lumbriculus variegatus*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20 - 25°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	300 mL minimum
9. Test solution volume:	100 mL minimum
10. Sediment depth:	3 cm
11 Renewal of test solutions:	None*
12. Age of test organisms:	Mixed age
13. No. organisms per test chamber:	10
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	50
16. Feeding regime:	10 mg trout chow starter on days 0, 5
17. Test chamber cleaning:	None
18. Test solution aeration:	None
19. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent, deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	1 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

Adapted from:

Ankley, G.T., R.A. Hoke, D.A. Benoit, E.N. Leonard, C.W. West, G.L. Phipps, V.R. Mattson and L.A. Anderson. 1993. Development and evaluation of test methods for benthic invertebrates and sediments: effects of flow rate and feeding on water quality and exposure conditions. Arch. Environ. Contam. Toxicol. 25:12-19.

ASTM. 1994. Method E1383-94. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Bailey, N.C. and D.N.W. Lui. 1980. *Lumbriculus variegatus*, a benthic oligochaete, as a bioassay organism. Pp. 202-215. In: J.C. Eaton, P.R. Parrish and A.C. Hendricks (Eds). *Aquatic Toxicology.* ASTM STP 707. American Society for Testing and Materials, Philadelphia, PA.

USEPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-94/024. U.S. Environmental Protection Agency, Duluth, MN.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE POLYCHAETE, *Neanthes arenaceodentata*, SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Static Renewal
2. Test duration:	28 d
3. Temperature:	20±1°C
4. Salinity:	20-35 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	12L/12D
8. Test chamber size:	1 L minimum
9. Test solution volume:	200 mL overlying water
10. Sediment depth:	2.5 cm (200 mL)
11. Renewal of test solutions:	Weekly
12. Age of test organisms:	2-3 wk
13. No. organisms per test chamber:	5 maximum
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	25 minimum
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms®, or equivalent, artificial seawater prepared with Millipore MILLI-Q®, or eqivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Bioaccumulation
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	8 L
25. Test acceptability criterion:	Adequate mass of organisms at test completion for detection of target analyte(s)

REFERENCES:

ASTM. 1994. Method E1611-94. Standard guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Dillon, T.M., D.W. Moore and A.B. Gibson. 1993. Development of a chronic sublethal bioassay for evaluating contaminated sediment with the marine polychaete worm, *Nereis (Neanthes) arenaceodentata.* Environ. Toxicol. Chem. 12:589-605.

Reish, D.J. 1992. Guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. ASTM Draft No. 5. July 3, 1992. American Society for Testing and Materials, Philadelphia, PA.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE POLYCHAETE, *Nereis virens*, SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Flow-through or Static Renewal
2. Test duration:	28 d
3. Temperature:	10 to 20°C
4. Salinity:	≥ 20‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D, 14L/10D, 12L/12D
8. Test chamber size:	1 L (beaker) or large chamber with multiple worms composited into a single replicate (e.g., 20 worms in 20 gallon aquarium)
9. Test solution volume:	> 750 mL/worm
10. Sediment depth:	≥ 4 cm
11. Renewal of test solutions:	Flow-through = 5-10 vol/d; Static Renewal = 3x/week
12. Age of test organisms:	adult (3 - 15g)
13. No. organisms per test chamber:	One per 1 L beaker, 20 per 20 gallon aquarium
14. No. replicate chambers per sediment:	5-8 (depending on desired statistical power)
15. No. organisms per sediment:	5-8 (assumes values to be determined on individuals)
16. Feeding regime:	None
17. Test chamber cleaning:	As needed
18. Test solution aeration:	Moderate, as needed
19. Dilution water:	Natural seawater or modified GP, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and control sediment
21. Dilution series:	N/A
22. Endpoint:	Bioaccumulation
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	200 mL per worm
25. Test acceptability criterion:	Adequate mass of organisms at test completion for detection of target analyte(s)

REFERENCE:

Lee II, H., B. Boese, J. Pelletier, M. Winsor, D. Specht and R. Randall. 1989. Guidance Manual: Bedded Sediment Bioaccumulation Tests. EPA/600/x-89/302. U.S. Environmental Protection Agency. 232 pp.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE POLYCHAETE, *Arenicola marina*, SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Flow-through or Static Renewal
2. Test duration:	28 d
3. Temperature:	10 to 20°C
4. Salinity:	≥ 25‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D, 14L/10D, 12L/12D
8. Test chamber size:	1-2 L
9. Test solution volume:	> 500 mL/beaker (e.g., four 1 L beakers in 8 L aquarium)
10. Sediment depth:	≥ 15 cm deep sediment (wet wt); minimum 400 g sediment (wet wt) plus 3.5 g sediment per g wet flesh weight per day (≤ 250 mm in grain size diameter)
11. Renewal of test solutions:	Flow-through = 5-10 vol/d; Static Renewal = 3x/week
12. Age of test organisms:	< 1 year (3-6 g wet weight, 5-10 cm length), larger organisms require more sediment, larger test chambers
13. No. organisms per test chamber:	One (1) per beaker maximum
14. No. replicate chambers per sediment:	5-8 (depending on desired statistical power)
15. No. organisms per sediment:	5-8 (assumes values to be determined on individuals)
16. Feeding regime:	None
17. Test chamber cleaning:	As needed
18. Test solution aeration:	Moderate, as needed
19. Dilution water:	Natural seawater or modified GP, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and control sediment
21. Dilution series:	N/A
22. Endpoint:	Bioaccumulation
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	1 L per treatment, minimum
25. Test acceptability criterion:	Adequate mass of organisms at test completion for detection of target analyte(s)

REFERENCES:

Lee II, H., B. Boese, J. Pelletier, M. Winsor, D. Specht and R. Randall. 1989. Guidance Manual: Bedded Sediment Bioaccumulation Tests. EPA/600/x-89/302. U.S. Environmental Protection Agency. 232 pp.

Gordon, D.C., J. Dale and P.D. Keiger. 1978. Importance of sediment-working by the deposit-feeding polychaete *Arenicola marine* on the weathering rate of sediment-bound oil. J. Fish Res. Bd. Canada. 35:591-603.

Huttel, M. 1990. Influence of the lugworm *Arenicola marina* on porewater nutrient profiles of sand flat sediments. Mar. Biol. Prog. Ser. 62:241-248.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE OLIGOCHAETE, *Lumbriculus variegatus*, SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Static Non-renewal* or Overlying Water Renewal
2. Test duration:	28 d
3. Temperature:	20 - 25°C
4. Salinity:	0 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	4 L minimum
9. Test solution volume:	1 L
10. Sediment depth:	3 cm
11 Renewal of test solutions:	Variable
12. Age of test organisms:	Mixed Age Adults
13. No. organisms per test chamber:	5 g (~500-1000) (Minimum)
14. No. replicate chambers per sediment:	4 minimum
15. No. organisms per sediment:	N/A
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
19. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent, deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Bioaccumulation
23. Sampling and sample holding requirements:	<6 wk
24. Sample volume required:	4 L
25. Test acceptability criterion:	Adequate mass of organisms at test completion for detection of target analyte(s)

REFERENCES:

Ankley, G.T., R.A. Hoke, D.A. Benoit, E.N. Leonard, C.W. West, G.L. Phipps, V.R. Mattson and L.A. Anderson. 1993. Development and evaluation of test methods for benthic invertebrates and sediments: effects of flow rate and feeding on water quality and exposure conditions. Arch. Environ. Contam. Toxicol. 25:12-19.

Phipps, G.L., G.T. Ankley, D.A. Benoit and V.R. Mattson. 1993. Use of the aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. Environ. Toxicol. Chem. 12:269-279.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (D.O.) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2).

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE MACOMA CLAM, *Macoma nasuta*, SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Flow-through or Static Renewal
2. Test duration:	28 d
3. Temperature:	12 - 16°C
4. Salinity:	≥ 25‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	12L/12D, 16L/8D, 10L/14D
8. Test chamber size:	250mL - 1 L (beaker)
9. Test solution volume:	> 750 mL/beaker (e.g., ten 250 mL beakers in 8L aquarium)
10. Sediment depth:	≥ 50 g wet wt sediment per g wet flesh (without shell)
11. Renewal of test solutions:	Flow-through = 5-10 vol/d; Static Renewal = 3 x/wk
12. Age of test organisms:	2 - 4 yr, 28 - 45 mm shell length
13. No. organisms per test chamber:	One (1) per beaker maximum
14. No. replicate chambers per sediment.:	5 - 8 (depending on desired statistical power)
15. No. organisms per sediment:	5 - 8 (assumes values to be determined on individuals)
16. Feeding regime:	None
17. Test chamber cleaning:	As needed
18. Test solution aeration:	Moderate, as needed
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Bioaccumulation
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	8 L
25. Test acceptability criterion:	Adequate mass of organisms at test completion for detection of target analyte(s)

REFERENCES:

Lee II, H., B. Boese, J. Pelletier, M. Winsor, D. Specht, and R. Randall. 1989. Guidance Manual: Bedded Sediment Bioaccumulation Tests. EPA/600/x-89/302. 232 pp.

Ferraro, S., H. Lee II, R. Ozretich, and D. Specht. 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. Arch. Environ. Contamin. Toxicol. 19:386-394.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE CLAM, *Yoldia limatula*, SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Flow-Through or Static Renewal
2. Test duration:	28 d
3. Temperature:	5 - 20°C (activity minimal at lowest temperature)
4. Salinity:	≥28‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	16L/8D, 14L/10D, 12L/12D
8. Test chamber size:	500 - 1000 mL (beaker)
9. Test solution volume:	>750 mL/beaker
10. Sediment depth:	100 - 300 g sediment (dry wt), depth greater than shell length. *Yoldia* actively resuspends sediments into water column, additional sediment may need to be added during test to maintain minimal sediment depth.
11 Renewal of test solutions:	Flow-through = 5-10 vol/d; Static Renewal = 3x/week
12. Age of test organisms:	1 - 2 cm g
13. No. organisms per test chamber:	One (1) per beaker
14. No. replicate chambers per sediment:	5 - 8 (depending on desired statistical power)
15. No. organisms per sediment:	5 - 8 (assumes values to be determined on individuals)
16. Feeding regime:	None
17. Test chamber cleaning:	As needed
18. Test solution aeration:	Moderate, as needed
19. Dilution water:	Natural seawater or modified GP, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent, or deionized water
20. Test concentrations:	Site sediment(s), a reference sediment, and control sediment
21. Dilution series:	N/A
22. Endpoint:	Bioaccumulation
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	1 L, minimum
25. Test acceptability criterion:	Adequate mass of organisms at test completion for detection of target analyte(s)

REFERENCES:

Lee II, H., B. Boese, J. Pelletier, M. Winsor, D. Specht and R. Randall. 1989. Guidance Manual: Bedded Sediment Bioaccumulation Tests. EPA/600/x-89/302. 232 pp. (ATS Deliverable).

Bender, K. and W.R. Davis. 1984. Effects of feeding on *Yoldia limatula* on bioturbation. Ophelia 23: 91-100.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Diporeia* sp., SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Static Non-renewal* or Overlying Water Renewal
2. Test duration:	28 d
3. Temperature:	4°C
4. Salinity:	0-20 ‰
5. Light quality:	Red darkroom light
6. Light intensity:	Low
7. Photoperiod:	Continuous
8. Test chamber size:	4 L minimum
9. Test solution volume:	to 4 L
10. Sediment depth:	3 cm
11 Renewal of test solutions:	Variable
12. Age of test organisms:	Mixed age juveniles
13. No. organisms per test chamber:	5 g (~500-1000) (minimum)
14. No. replicate chambers per sediment:	4 minimum
15. No. organisms per sediment:	N/A (>10g OC/g organism)
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
19. Dilution water:	Moderately hard water; synthetic water modified to reflect receiving water hardness or salinity to 20%
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Bioaccumulation
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	8 L
25. Test acceptability criterion:	Adequate mass of organisms at test completion for detection of target analyte(s)

REFERENCES:

Landrum, P.F. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod, *Pontoporeia hoyi*. Environ. Sci. Technol. 23:588-595.

Landrum, P.F., B.J. Eadie and W.R. Faust. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* spp. Environ. Toxicol. Chem. 10:35-46.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (D.O.) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2).

APPENDIX F
METHODOLOGIES FOR IDENTIFYING AMMONIA AS A TOXICANT IN DREDGED-MATERIAL TOXICITY TESTS

APPENDIX F

Ammonia Toxicity: General Overview

Ammonia is a relatively toxic compound which, in sediments, is generated from the microbial degradation of nitrogenous organic material such as amino acids (Santschi et al., 1990). Resulting interstitial (pore) water concentrations of ammonia in otherwise uncontaminated sediments can be as high as 50 mg/L (Murray et al., 1978; Kristensen and Blackburn, 1987), while ammonia concentrations in pore water from contaminated sediments may range from 50 to greater than 200 mg/L (Ankley et al., 1990; Schubauer-Berigan and Ankley, 1991). Hence, exposure of epibenthic/benthic test species to ammonia in solid phase tests can be significant. Moreover, because ammonia is released from sediments relatively readily during resuspension events (Blom et al., 1976), high concentrations can also occur in test elutriates. Both marine and freshwater studies suggest that ammonia can be responsible for toxicity observed in some laboratory sediment toxicity tests (Jones and Lee, 1988; Ankley et al., 1990).

Because ammonia is not extremely persistent, its toxicity may not be of as much concern as that from, for instance, metals or pesticides. For this reason, there has been a tendency in some situations to use open-water disposal for dredged material whose toxicity is suspected to be due to ammonia. Unfortunately it has previously been difficult, if not impossible, to validly link sediment or elutriate toxicity to ammonia when multiple sediment contaminants are present (Ankley et al., 1992), in particular because ammonia concentrations can be exceptionally high in sediments which are also toxic due to other, persistent contaminants such as inorganic and/or organic chemicals (Schubauer-Berigan and Ankley, 1991). However, recent technical developments have resulted in a logical conceptual framework, specifically a simple risk assessment, for deciding whether observed sediment (or elutriate) toxicity may be due to ammonia. Briefly, data are collected on the toxicity of ammonia to the test species of concern (effects assessment), and concentrations of ammonia are measured in appropriate test fractions (elutriate, overlying water, pore water) during the toxicity test (exposure assessment). If concentrations of ammonia in the test are large enough to result in toxicity to the test species of concern (risk characterization), a simple set of toxicity identification evaluation (TIE) procedures is next used to confirm that toxicity is indeed due to ammonia and not to other contaminants in the sediment (Ankley et al., 1992). TIE methods consist of physical/chemical sample manipulations conducted concurrently with toxicity testing in order to directly characterize and identify contaminants responsible for toxicity in complex mixtures. Further information on how this approach could be used, and important technical considerations relative to this assessment are described below.

Specific Considerations for Assessing Ammonia Toxicity in Dredged Material

The first step in assessing the potential for ammonia toxicity in a sediment test is to routinely measure ammonia concentrations in test fractions of concern, at a minimum, when starting and ending the test. Due to the influence of pH on ammonia toxicity to some species, it is essential that pH also be measured and recorded simultaneously. For elutriate tests, ammonia measurements can be made on whole elutriate. For solid phase tests, ammonia should be measured both in overlying water and in pore water, the potential routes of ammonia exposure for epibenthic and benthic species. In tests where periodic renewal of overlying water is utilized, ammonia may not be present at toxicologically significant concentrations in the overlying water (Ankley et al., 1993); nonetheless, it would still be prudent to measure ammonia in the overlying water. Regardless of whether overlying water renewal is used in a sediment test, pore water ammonia concentrations should be determined. Pore water for ammonia measurements can be isolated using any of a variety of techniques (e.g., low-speed centrifugation, squeezing, peepers, etc.). Unlike other pore water contaminants of concern (e.g., metals, nonionic organics), it does not appear that the method used to isolate pore water greatly affects observed ammonia concentrations (EPA, 1991a). Upon isolation of the appropriate test fraction(s), ammonia can be measured using any accepted technique; specific ion electrodes are rapid, simple and often used for ammonia determinations at concentrations $\geq$ 1 mg/L (EPA, 1979).

The next step is to compare exposure data (i.e., ammonia measurements) to toxicity data. The basis of this comparison most generally will be to ammonia toxicity data generated in water-only toxicity tests. For the elutriate tests the comparison can be made directly while, for solid phase tests, the water-only toxicity data are compared to overlying water and/or pore water ammonia concentrations. To assess the potential for ammonia toxicity in a test with a given species, it is essential that comparisons be made to toxicity data generated with that same species in tests conducted under conditions reasonably similar to the sediment test. The tendency to attempt to extrapolate toxicity data for one species to another species should be avoided. Such an approach may be appropriate for some types of risk analyses; however, for the approach described here, this type of extrapolation likely would result in erroneous conclusions. Similarly, comparisons within a species should be made only between tests which were conducted under a relatively similar set of conditions. For example, it would be inappropriate to compare toxicity data and ammonia concentrations from a short-term sediment test to water-only chronic toxicity data for that same species. In addition to test length, pH is of primary concern while hardness, salinity and temperature are of somewhat lesser concern. All of these factors can markedly influence ammonia toxicity, and must be accounted for to enable among test comparability.

Although there is a good deal of data on the toxicity of ammonia to various aquatic species (EPA, 1985), much of this information was generated using pelagic species (e.g., cladocerans, fishes), which precludes comparison to sediment exposures with commonly tested benthic species (e.g., amphipods). [Although it should be noted that these data would be useful for extrapolation to elutriate tests which commonly utilize pelagic species].

Water-only toxicity data are available for some epibenthic/benthic species of concern, however these water-only tests were often conducted under conditions quite different from those commonly used in sediment tests, which greatly limits any extrapolation to sediment tests. However, efforts are now underway to generate useful data. For example, toxicity data now exist for ammonia at four different pHs (ca., 6.5, 7.2, 7.8, 8.6) for *Hyalella azteca, Chironomus tentans* and *Lumbriculus variegatus* (EPA, 1991a; G. Ankley, unpublished data). Ammonia toxicity data have also been developed for the commonly tested marine amphipods *Rhepoxynius abronius*, *Eohaustorius estuarius*, *Ampelisca abdita* and *Grandidierella japonica* in four-day water-only exposures at a pH of 8.0 (Kohn et al., 1993); and, ammonia toxicity data have been generated for the polychaete *Nereis (Neanthes) arenaceodentata* (Dillon et al., 1993).

Although toxicity data exist for several pelagic and some benthic species of concern, it may be necessary for laboratories conducting dredged material tests with a particular species, under a given set of test conditions, to develop ammonia toxicity data relevant to their species/test conditions. This likely would be a wise investment of resources, in particular for those laboratories conducting large numbers of tests with dredged material.

In this regard, a major caution must be noted concerning pH in ammonia tests. Ammonia acts as a basic compound in water. The un-ionized form (NH_3) predominates at pH values greater than 9.3, while the ionized form (NH_4^+) is most abundant at pH values less than 9.3. Through the pH range of 6 to 8.3 (which is typically encountered in freshwater and marine sediment tests), the percentage of un-ionized ammonia changes approximately 250-fold. Based on models developed primarily with fish, it has been common to express ammonia toxicity data on an un-ionized (i.e., NH_3) rather than a total (i.e., NH_3 plus NH_4^+) basis. This implicitly suggests that ionized ammonia is not of great toxicological significance. While this appears to be true for fish (EPA, 1985), it does not appear to be the case for some invertebrates. For example, *H. azteca* displays the same sensitivity to total ammonia (NH_3 plus NH_4^+) over a pH range of approximately 6.0 to 8.5, suggesting that this amphipod is very sensitive to ammonium ion (EPA, 1991a; G. Ankely, unpublished data). Hence, extrapolation of ammonia toxicity data collected at only one pH value, based on un-ionized ammonia concentrations, would result in inaccurate predictions of potential toxicity of ammonia to at least this amphipod. Other invertebrates may exhibit a similar lack of predictability relative to pH/ammonia interactions. Unfortunately, relatively few ammonia toxicity tests with invertebrates have been conducted at multiple pHs; thus, it is difficult to broadly predict responses to ammonia at different pH values. To make accurate predictions of potential ammonia toxicity for a particular test species, it is important to obtain (or generate) ammonia toxicity data within the pH range in which extrapolations are made.

If measurements of ammonia in elutriate tests, or overlying and/or pore water in solid phase tests are determined to be of possible toxicological significance, it is essential that the role of ammonia in causing toxicity be confirmed. It is important to avoid the tendency to assume that if a dredged material test exhibits

toxicity and ammonia is present, that ammonia is the sole (or even major) cause of the observed toxicity. Toxic concentrations of other contaminants may be present simultaneously with ammonia. In such cases the assumption that only ammonia was causing toxicity could lead to disposal decisions (i.e., open-water) that may result in serious long-term impacts to benthic communities.

Relatively simple TIE manipulations as generally described by Ankley et al. (1992), and specifically in a series of guidance manuals (EPA, 1988; 1989a; 1989b; 1991a; 1991b) may be used to determine whether (or not) ammonia is responsible for the observed toxicity. To date, these TIE methods have only been used with freshwater sediments. However, in many instances similar approaches can be used with marine sediments; also, EPA currently is developing standardized TIE methods for marine sediments.

Current sediment TIE methods are only for elutriates or pore waters and for short-term (≤ 96 hour) tests (EPA, 1991a). This is not a problem if TIE procedures are to be used with toxic elutriates, because elutriate tests also generally consist only of short-term (≤ 96 hour) exposures. However, solid phase tests with dredged material are generally 10 days in length. Although using pore water as a surrogate test fraction for TIE work with solid phase exposures could mean that toxicity might not be expressed in the shorter-term pore water exposures, this may not be a significant problem in the case of ammonia. In water-only exposures with three different benthic invertebrates (*H. azteca, C. tentans, L. variegatus*), the majority of toxicity due to ammonia was observed within 4 days in a 10 day test (G. Ankley, unpublished data).

There is one other important consideration relative to the use of pore water as a surrogate test fraction for solid phase sediments. Because the toxicity of ammonia to some organisms can be pH-dependent, it is imperative that pH in pore water tests mimic the pH in the initial solid phase tests. This is particularly important with freshwater sediments, because pH can drift upwards by as much as one unit over the course of a 96-hour test (Ankley et al., 1991). Methods which have proven useful for controlling pH in pore water tests include: (a) use of acids/bases in chambers with minimal head-space, (b) use of organic buffers, and (c) use of varying amounts of CO_2 in head-space overlying the pore water (EPA, 1991a; 1991b).

Most aquatic species that can be tested successfully in a water-only exposure can be utilized for TIE work. For example, cladocerans (*Ceriodaphnia dubia, Daphnia magna, Daphnia pulex*), fish (*Pimephales promelas, Oryzias latipes, Oncorhynchus mykiss*), amphipods (*H. azteca*), oligochaetes (*L. variegatus*), and chironomids (*C. tentans*) all have been used for freshwater TIE studies. The best choice of a TIE organism is, of course, the same species that was sensitive to the original elutriate or solid phase sediment of interest. For example, if toxicity was observed in solid phase sediment tests with *H. azteca*, that species would be the best choice for pore water TIE work. Of course, there are instances in which this may not be possible; for example, the test species of concern may be of limited availability. In this case, it may be possible to use surrogate species for the TIE, provided there is adequate knowledge of the sensitivity of the surrogate species to ammonia, relative to the original test

species of concern. If a surrogate species is used, upon conclusion of the TIE it is important to perform limited testing to confirm that the same compound(s) which was toxic to that species was responsible for toxicity to the original test species.

As discussed above, the toxicity of ammonia to many species can be highly pH-dependent. If the test species of concern is more sensitive to un-ionized that ionized ammonia, samples will be more toxic at high pH values than at low pHs. [Note that this again demonstrates the need for data concerning pH/ammonia interactions for specific test organisms]. If the test species exhibits this pH-dependency with regard to ammonia toxicity, the graduated pH test can be an extremely powerful tool for implicating ammonia as a suspect toxicant. The graduated pH test is conducted at a series of physiologically tolerable pHs (generally ranging from 6.0 to 8.5); if sample toxicity is greater at higher pH values, this suggests that ammonia is responsible for at least some of the observed toxicity. A number of other TIE techniques also exist for implicating ammonia. These include evaluation of relative species sensitivity (e.g., fish are generally more sensitive than cladocerans), removal of ammonia from the test samples with cation exchange resins (e.g., zeolite) and/or extended air-stripping at elevated pH values (e.g., >10) prior to toxicity testing, correlation of toxicity with measured ammonia concentrations and toxicity tests at different pH values with equitoxic concentrations of ammonia (EPA, 1989a; Ankley et al., 1990). Another useful method for confirming that ammonia is responsible for toxicity is ammonia removal followed by spiking to restore the original ambient concentrations of ammonia. The spiked sample is then tested for toxicity; if ammonia is the causative toxicant, observed toxicity theoretically should be the same as that observed in the original sample. It is desirable to conduct as many of these confirmation tests as possible because no single test is specific for ammonia, e.g., zeolite will remove cationic metals, as well as ammonia, from test samples. Failure of one or more of the tests to confirm ammonia as responsible for toxicity would indicate that other contaminants were contributing to sample toxicity.

Summary

In order to identify elutriate or solid phase dredged material toxicity due to ammonia, it is essential to make routine measurements of ammonia on appropriate test fractions. These measurements then are compared to water-only toxicity data for the same species used in the dredged material test. The water-only toxicity data should be generated under conditions (e.g., pH, test length) reasonably similar to those in the test with the dredged material. If ammonia concentrations are too low to have potentially caused the observed toxicity in the dredged material sample, other contaminants are responsible for the toxicity. If ammonia concentrations are high enough to have caused the observed toxicity, TIE procedures should be used to confirm this suspicion. When there is no TIE confirmation that ammonia is responsible for sediment toxicity, it must be assumed that persistent contaminants other than ammonia are causing toxicity.

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APPENDIX G
QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC) CONSIDERATIONS

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G.0 QUALITY ASSURANCE/QUALITY CONTROL (QA/QC) CONSIDERATIONS

G.1 Introduction

The following sections provide guidance for QA/QC. More detailed guidance, pertaining largely to physical and chemical evaluations, is provided in EPA (1995). This new QA document is applicable to both the Inland Testing Manual and to the Ocean Disposal "Green Book" (EPA/USACE, 1991), and will: 1) provide guidance on the development of QA project plans for ensuring the reliability of data gathered to evaluate dredged material proposed for discharge under the CWA or the MPRSA; 2) outline procedures that need to be followed when sampling and analyzing sediments, water, and tissues; and 3) provide recommended target detection limits (TDLs) for chemicals of concern.

A quality assurance (QA) program integrates management and technical practices into a single system to guarantee quality environmental data. The purpose of a QA program in a dredged material evaluation is to provide environmental data that are sufficient, appropriate, and of known and documented quality. Major elements of a QA program are:

- human resource training
- QA management plan (QAMP)/QA project plan (QAPP)
- management system reviews
- data quality objectives (DQOs)
- standard operating procedures (SOPs)
- project specific technical assessments.

QA project plans provide, in one place, a detailed plan for the activities performed at each stage of the dredged material evaluation (including appropriate sampling and analysis procedures) and outline project-specific data quality objectives that should be achieved for field observations and measurements, physical analyses, laboratory chemical analyses, and biological tests. Data quality objectives must be defined prior to initiating a project and adhered to for the duration of the project in order to guarantee acquisition of reliable data. This is accomplished by integrating quality control (QC) into all facets of the project, including development, implementation, and evaluation. QC is the routine application of procedures for determining bias and precision. QC procedures include activities such as preparation of replicate samples, spiked samples, blanks; calibration and standardization; sample custody and recordkeeping. Audits, reviews and compilation of complete and thorough documentation are activities used to verify compliance with pre-defined QC procedures. Through periodic reporting, these activities provide a means for management to track project progress and milestones, performance of measurement systems, and data quality.

A complete QA/QC effort for a dredged material testing program has two major components: a QA program implemented by the responsible governmental agency (the data user), and QC programs implemented by sampling and laboratory personnel performing the tests (the data generators). QA programs are also implemented by each field contractor and each laboratory. Typically, all field and laboratory data generators agree to adhere to the QA/QC of the data user for the contracted project as specified in the project QAPP. EPA (1987) provides useful guidance and may be followed on all points that are not in conflict with the guidance in this manual.

G.1.1 Government (Data User) Program

The USACE must implement a QA program to ensure that all program elements and testing activities (including field and laboratory operations) in the dredged material evaluation comply with the procedures in the QA project plan or with other specified guidelines for the production of environmental data of known quality. QA oversight is the responsibility of the USACE District Office, working in conjunction with the EPA Region. USACE Districts are responsible for ensuring that both the data submitted with permit applications, and that laboratories under contract to their Districts comply with the QA needs of the regulations and guidelines governing dredged material evaluations. The QA program should be designed with the assistance of programmatic and scientific expertise from both EPA and USACE. Other qualified sources of QA program management should be contacted as appropriate. Some specific QA considerations in contract laboratory selection are discussed by Sturgis (1990) and EPA (1991a).

G.1.2 Contractor (Data Generator) Program

Each office or laboratory participating in a dredged material evaluation is responsible for using procedures which assure that the accuracy (precision and bias), representativeness, comparability, and completeness of its data are known and documented. To ensure that this responsibility is met, each participating organization should have a project manager and a written QA management plan that describes, in specific terms, the management approach proposed to assure that each procedure under its direction complies with the criteria accepted by EPA and USACE. This plan should describe a QA policy, address the contents and application of specific QA project plans, and specify training requirements. All field measurements, sampling, and analytical components (physical, chemical, and biological) of the dredged material evaluation should be discussed.

For the completion of a dredged material testing project, the project manager of each participating organization should establish a well-structured QA program that ensures the following:

- development, implementation, and administration of appropriate QA planning documents for each study
- inclusion of routine QC procedures for assessing data quality in all field and laboratory standard operating procedures (SOPs)
- performance of sufficiently detailed audits at intervals frequent enough to ensure conformance with approved QA project plans and SOPs
- periodic evaluation of QC procedures to improve the quality of QA project plans and SOPs
- implementation of appropriate corrective actions in a timely manner.

G.2 The QA Project Plan

The QA project plan should be developed by the applicant or contractor for each dredged material evaluation, in accordance with EPA (1995). The QA project plan provides an overall plan and contains specific guidelines and procedures for the activities performed at each stage of the dredged material testing program, such as dredging site subdivision, sample collection, bioassessment procedures, chemical and physical analyses, data quality standards, data analysis and reporting. In particular, the QA plan addresses required QC checks, performance and system audits, QA reports to management, corrective actions, and assessment of data accuracy (precision and bias), representativeness, comparability and completeness. The plan should address the quantity of data required to allow confident and justifiable conclusions and decisions. QA project plans are particularly useful for work that involves many people or for projects that continue 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 for 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.

Each of the following items should be considered for inclusion in the QA Project Plan:

- Project description (G.2.1)
- QA organization; personnel responsibilities and qualifications (G.2.2)
- QA objectives for measurement data in terms of accuracy, representativeness, comparability, and completeness (G.2.3)

- Standard operating procedures (G.2.4)
- Sampling strategy and procedures (G.2.5)
- Sample custody and documentation (G.2.6)
- Calibration procedures (G.2.7)
- Analytical procedures (G.2.8)
- Data validation, reduction and reporting (G.2.9)
- Internal QC checks (G.2.10)
- Performance and system audits (G.2.11)
- Facilities (G.2.12)
- Preventative maintenance (G.2.13)
- Calculation of data quality indicators (G.2.14)
- Corrective actions (G.2.15)
- QA reports to management (G.2.16).

G.2.1 Project Description

A project description should be provided that defines project goals and illustrates how the project will be designed to obtain the information needed to achieve those goals. Sufficient detail and information should be included to allow decisions during the joint EPA and USACE review and the final USACE approval phases. Where appropriate, the following information should be included in this section of the QA project plan:

- objectives and scope of the project
- any historical information relevant to the dredging operation
- intended activities further described in flow diagrams, tables, and charts
- schedule of tasks and milestones
- intended use of acquired data.

G.2.2 QA Organization; Personnel Responsibilities and Qualifications

A clear delineation of the QA organization and line of authority is essential for the development, implementation, and administration of a QA program. This should include all technical personnel, including key individuals responsible for ensuring sufficient QC is being incorporated into the project. Organizational charts or tables should be used in the QA project plan to describe the management structure, personnel responsibilities, and the interaction among functional units. Each QA task should be

fully described and the responsible individual and associated organization named. An example of a QA organization flow diagram is provided in Appendix G.4.

Technical staff are responsible for the validity and integrity of the data produced. The QA staff should be responsible for ensuring that all personnel performing tasks related to data quality are appropriately qualified. Records of qualifications and training of personnel should be kept current for verification by internal QA personnel or by EPA and USACE.

G.2.3 Data Quality Objectives

Data quality objectives are used to ensure that the data are acceptable. They define performance-based goals for accuracy (precision and bias), representativeness, comparability, and completeness as well as the required sensitivity of chemical measurements (i.e., target detection limits, TDLs). Accuracy is defined in terms of bias (how close the measured value is to the true value) and precision (how variable the measurements are when repeated). Data quality objectives should be based on the intended use of the data, technical feasibility, and consideration of cost. Numerical quality objectives should be summarized in a table, with all data calculated and reported in units consistent with other organizations reporting similar data, to allow comparability of data bases. All measurements should be made so that results are representative of the medium (e.g., water, sediments, tissue) being measured. Data quality objectives for precision and bias established for each measurement parameter should be based on prior knowledge of the measurement system employed, method validation studies, and the requirements of the specific project. An example of a data quality objectives summary for laboratory measurements is provided in Appendix G.4.

G.2.4 Standard Operating Procedures

Standard operating procedures (SOPs) are written descriptions of routine methods and should be provided for as many methods used during the dredged material evaluation as possible. A large number of field and laboratory operations can be standardized and presented as SOPs. Once these procedures are specified, they can be referenced or provided in an appendix of the QA project plan. Only modifications to SOPs or non-standard procedures need to be explained in the main body of the QA project plan (e.g., in the "sampling procedures" or "analytical procedures" section). General types of procedures benefiting from SOPs are field measurements ancillary to sample collection (e.g., depth of overlying water, sampling depth, water quality measurements, mixing model input measurements), chain-of-custody, sample handling and shipment, and routine analytical methods for chemical analyses. SOPs ensure that all persons conducting work are following the same procedures and that the procedures do not change

over time. All personnel should be thoroughly familiar with the SOPs before work is initiated. Deviations from SOPs may affect data quality and integrity. If it is necessary to deviate from approved SOPs, these deviations must be documented and approved through an appropriate chain-of-command which may include USACE and EPA. Personnel responsible for ensuring the SOPs are adhered to must be identified in the QA Project Plan. Example SOPs are provided in Appendix D of EPA (1995).

G.2.5 Sampling Strategy and Procedures

A sampling strategy should be developed to ensure that the sampling design supports the planned data use. The sampling strategy will strongly affect the representativeness, comparability, and completeness that might be expected for field measurements. In addition, the strategy for collecting field QC samples (e.g., replicates) will assist in the determination of how well the total variability of a field measurement can be documented. Therefore, development of the sampling strategy should be closely coordinated with development of data quality objectives discussed in Section G.2.3.

To reduce sampling error, all methods, procedures, and equipment to be used in the field should be documented in a sampling plan which has been authorized and which is readily available to all personnel. The purpose of this sampling plan is to provide a blueprint for all field work by defining in detail the appropriate sampling and data collection methods (in accordance with the established data quality objectives). Written procedures or checklists for field equipment, sample container preparation, sample preservation, labelling and numbering systems, and shipping procedures must be appropriate. Methods to record and report deviations from the sampling plan must also be described. An alteration checklist form is generally appropriate to implement required changes. An example of such a checklist is provided in Appendix G.4.

G.2.6 Sample Custody and Documentation

Sample custody and documentation are vital components of all dredged material evaluations, particularly if any of the data may be used in a court of law. It is important to record all events associated with a sample so that the validity of the resulting data may be properly interpreted. Documentation is necessary during the field effort when samples are collected and in the laboratory where both chemical and biological analyses are performed. Thorough documentation provides a means to track samples from the field through the laboratory and prevent sample loss. The contents and location of all documents related to dredged sediment samples should be specified, and access to the samples should be controlled. Where samples may be needed for potential litigation, chain-of-custody procedures should be followed. Chain-of-custody procedures are initiated during sample collection. They include a descriptive label and

tracking report forms for both the field and laboratory. An example of a label, field tracking report form, laboratory tracking report form and chain-of-custody record is provided in Appendix G.4.

G.2.6.1 Field Operations

The potential for sample deterioration and/or contamination exists during sample collection, handling, preservation, and storage. Approved protocols and SOPs should be followed to ensure all field equipment is acceptably calibrated and to prevent deterioration or contamination. Experienced personnel should be responsible for maintaining the sample integrity from collection through analysis. A complete record of all field procedures, an inventory log, and a tracking log should be maintained. A field tracking report should identify sample custody and conditions in the field prior to shipment.

Dates and times of collection, station locations, sampling methods, and sample handling, preservation, and storage procedures should be documented immediately, legibly, and indelibly so that they are easily traceable. Any circumstances potentially affecting sampling procedures should be documented. The data recorded should be thorough enough to allow station relocation and sample tracking. An example of a station location log is provided in Appendix G.4. Any field preparation of samples should also be described. Samples should be identified with a pre-prepared label containing at least the following information:

- project title
- sample identification number
- location (station number) and depth
- analysis or test to be performed
- preservation and storage method
- date and time of collection
- special remarks if appropriate
- initials of person collecting the sample
- name of company performing the work.

G.2.6.2 Laboratory Operations

The responsible party who will act as sample custodian at the laboratory facility should be identified. This individual has authority to sign for incoming field samples and has the responsibility to obtain documents of shipment and verify the data entered on the sample custody records. A laboratory-tracking report should be prepared for each sample. The location of samples processed through chain-of-custody

must be known at all times. Samples to be used in a court of law must be stored in a locked facility to prevent tampering or alteration.

A procedure should be established for the retention of all appropriate field and laboratory records and samples as various tasks or phases are completed. Replicates, subsamples of analyzed samples, or extra unanalyzed samples should be kept in a storage bank. These samples can be used to scrutinize anomalous results or for supplemental analyses, if additional information is needed. All samples should be properly stored and inventoried. The retention and archiving procedure should indicate the storage requirements, location, indexing codes, retention time, and security requirements for samples and data.

G.2.7 Calibration Procedures

Calibration procedures should be included for each instrument used during the study. The appropriate procedures used to assure that field and laboratory equipment are functioning properly should be documented in this section. This information can be provided in tabular format. The planned frequency for recalibration should be provided as well as a list of the calibrations standards to be used and their sources, including traceability procedures. Instrumentation that requires routine calibration includes, for example, navigation devices, analytical balances, and water quality meters.

G.2.8 Analytical Procedures

The methods cited in the analytical procedures section of a QA project plan are used to meet the data quality objectives for a dredged material evaluation. (Section 9 of this Manual provides guidance on the selection of physical and chemical analyses to aid in evaluating dredged material proposed for disposal, and on the methods used to analyze these parameters.) In all cases, proven, state-of-the-art methods should be used. Sample analysis procedures are identified in this section of the QA project plan by reference to established, standard methods. Any modifications to established, standard methods and any specialized, nonstandard procedures should be described in detail in this section of the plan.

G.2.9 Data Validation, Reduction and Reporting

Data validation involves all procedures used to accept or reject data after collection and prior to use. These include screening, editing, verifying, and reviewing through external performance evaluation audits. Data validation procedures ensure that objectives for data precision and bias were met, that data were generated in accordance with the QA project plan and SOPs, and that data are traceable and

defensible. All data should be reported with their associated analytical sensitivity, precision, and bias. In addition, the level of quantification achieved by the laboratory should be compared to specific target detection limits. The following information should be included in the QA project plan:

- the principal criteria that will be used to validate data integrity during their collection and reporting
- the data reduction scheme planned for collected data including all equations used to calculate the concentration or value of the measured parameter and reporting units
- the methods used to identify and treat outliers and nondetectable data
- the data flow or reporting scheme from collection of raw data through storage of validated concentrations (a flowchart is usually necessary)
- statistical formulae and sample calculations planned for collected data
- key individuals who will handle the data in this reporting scheme.

QC procedures designed to eliminate errors during the mathematical and/or statistical reduction of data should also be included in the QA project plan. Quality control in data processing may include both manual and automated review. Input data should be checked and verified to confirm compatibility and to flag "outliers" for confirmation. Computerized data plots can be routinely used as a tool for rapid identification of outliers that can then be verified using standard analytical procedures.

Data entries should be dated when entered, and signed or initialled by the person making the measurement and the person entering the data. Changes to entries should be made so as not to obscure the original entry. They 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.

The data and information collected during the Tier I evaluation should be carefully reviewed as to their relevancy, completeness, and quality. The data must be relevant to the overall objective of the project, even though the objectives for these studies were different.

G.2.10 Internal Quality Control Checks

The various control samples that will be used internally by the laboratory or sample collection team to assess quality are described in this section of the QA project plan. For most environmental investigations, 10-30 percent of all samples may be analyzed specifically for purposes of quality control. In some special cases (e.g., when the number of samples is small and the need to establish the validity of analytical data is large), as many as 50 percent of all samples are used for this purpose. These QC samples may be used to check the bias and precision of the overall analytical system and to evaluate the performances of individual analytical instruments or the technicians that operate them. The most widely used QC samples are summarized in EPA (1995) and are as follows:

- blanks
- matrix spike samples
- surrogate spike compounds
- check standards, including:
  - spiked method blanks
  - laboratory control samples
  - reference materials
- matrix replicates (split in the laboratory from one field sample)
- field replicates (collected as separate field samples from one location).

The following sections discuss quality control procedures for sediment, water, and tissue analyses (see EPA, 1995 for further detail), as well as for biological analyses.

The USACE District 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 USACE may participate in some studies. These activities provide an independent quality assurance check on activities being performed and on data being generated and are discussed in Section G.2.11.

G.2.10.1 Quality Control Considerations for Physical Analysis of Sediments

The procedures used for the physical analysis of sediments must include a QC component. QC procedures for grain-size analysis and total solids/specific gravity determinations are necessary to ensure that the data meet acceptable criteria for precision and bias. To measure precision, triplicate analyses should be performed for every 20 samples analyzed. TOC is a special case, where all samples should be analyzed 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 must be verified by independent check standards to confirm the bias of the results. QC limits should be agreed upon for each analytical procedure, and should be consistent with the overall QA project plan.

G.2.10.2 Quality Control Considerations for Chemical Analysis of Sediments

Methods for the chemical analysis of contaminants of concern in sediments must include detailed procedures and requirements which should be followed rigorously throughout the evaluation. General procedures include the analysis of a procedural blank, a matrix duplicate, a matrix spike along with every 10 - 20 samples processed, and surrogate spike compounds (for organic analyses only). All analytical instruments should be calibrated at least daily. All calibration data should be submitted to the laboratory project QA coordinator for review. The QA/QC program must document the ability of the selected methods to address the high salt content of sediments from marine and estuarine areas.

Analytical precision can be measured by analyzing one sample in duplicate or triplicate for every 10 - 20 samples analyzed. If duplicates are analyzed, the relative percent difference should be reported. However, if triplicates are analyzed, the percent relative standard deviation should be reported.

G.2.10.3 Quality Control Considerations for Chemical Analysis of Water

Methods recommended for the chemical analysis of contaminants of concern in water include detailed QC procedures and requirements which should be followed closely throughout the evaluations. General procedures should include the analysis of a procedural blank, a matrix duplicate, a matrix spike for every 10 - 20 samples processed, and surrogate spike compounds (for organic analysis only). Analytical precision can be measured by analyzing one sample in triplicate or duplicate for every 10 - 20 samples analyzed. If duplicates are analyzed, the relative percent difference should be reported. However, if triplicates are analyzed, the percent relative standard deviation should be reported. Analytical bias can be measured by analyzing standard reference materials (SRMs), a matrix containing a known amount of a pure reagent. Recoveries of surrogate spikes and matrix spikes should be used to measure for precision

and bias; results from these analyses should be well documented. Special QC is required for ICP and GC/MS analyses. Initial calibrations using three or five standards (varying concentrations) are required before analyzing samples. Subsequent calibration checks should be performed for every 10 - 20 samples analyzed.

G.2.10.4 Quality Control Considerations for Chemical Analysis of Tissue

As with sediments and water, methods recommended for the chemical analysis of contaminants of concern in tissues include detailed QC procedures and requirements which should be followed closely throughout the evaluations. General procedures should include the analysis of a procedural blank, a matrix duplicate, a matrix spike for every 10 - 20 samples processed, and surrogate spike compounds (for organic analyses only). Analytical precision can be measured by analyzing one sample in triplicate or duplicate for every 10 - 20 samples analyzed. If duplicates are analyzed, the relative percent difference should be reported. However, if triplicates are analyzed, the percent relative standard deviation should be reported. Analytical bias can be measured with the appropriate SRMs. Precision and bias determinations should be performed with the same frequency as the blanks and matrix spikes.

G.2.10.5 Quality Control Considerations for Biological Analyses

Quality controls for tests of biological effects and bioaccumulation must address all activities that affect the quality of the data (e.g., see EPA, 1991b). These activities include:

- source and condition of test organisms
- use of negative (non-toxic) and positive (reference toxicants) controls
- acceptability of test results and data evaluation.

Standard laboratory procedures must be followed in all testing including maintenance/measurement of environmental (e.g., water) quality conditions and blind testing.

G.2.10.5.1 Source and Condition of Test Organisms

Test organisms should be positively identified to species by qualified experts. Test organisms should appear healthy, behave normally, feed well, and have low mortality in cultures, during holding, and in

test controls. The quality of test organisms from outside sources as well as those maintained in-house must be verified by conducting a reference toxicant test concurrently with the dredged material toxicity tests. The supplier should provide data with the shipment describing the history of the sensitivity of organisms from the same source culture, determined in monthly tests using suitable reference toxicants.

G.2.10.5.2 Reference Toxicants

Biological QC includes periodic reference toxicant tests with all stocks of organisms to be used in testing to determine the relative health of the 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 EPA or USACE.

Reference toxicants are routinely used to evaluate species sensitivity, laboratory performance and both intra- and inter- laboratory precision. The following chemicals provide good endpoints for a variety of species: freshwater species - sodium chloride, copper sulfate, potassium chloride, cadmium chloride, sodium dodecyl sulfate, diazinon; saltwater species - copper sulfate, cadmium chloride, sodium dodecyl sulfate, diazinon. It is required that a set of the above chemicals with difference modes of toxic action be used as reference toxicants in establishing comparative sensitivity between recommended species listed in Tables 11, 12 and 13 of the Manual and a species proposed as a substitute regional test species.

Reference toxicant tests should be performed routinely on all groups of organisms used in dredged material toxicity and bioaccumulation studies in order to determine their relative health and vigor. A single reference toxicant can be used to assess this in routine testing. Many chemicals may be used satisfactorily as reference toxicants (e.g., Lee, 1980; Wang, 1987; EPA, 1990, 1991c). Reference toxicant tests are performed in the absence of sediment and generally under static conditions. Water-only reference toxicant tests with benthic species may require some modification to "standard" test conditions used for pelagic species. A short term response to a standardized exposure is used as an indication of the relative health of the organisms. A geometric dilution series of five unreplicated concentrations is used plus a negative (dilution-water only) control. Although nominal concentrations are usually sufficient for reference toxicant tests, concentrations should be measured whenever possible. 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 range-finding test using a very wide range of concentrations may be necessary to determine the proper concentration range for reference toxicant tests. For each species, mortality is determined and the LC_{50} or EC_{50} is calculated as described in Appendix D.

A control chart should be developed for each reference toxicant/test organism combination used (e.g., see EPA, 1990). The LC_{50} or EC_{50} for each combination should be determined on a regular basis, and each combination tracked on a separate Average Control Chart (Figure G.1). Successive toxicity values should be plotted and examined to determine if the results are within the established limits. Commonly used limits are the mean±2 standard deviations. A minimum of five data points are necessary to develop the first set of limits. These limits are recalculated for each successive data point, until the statistics stabilize. Organisms are suitable for dredged material testing if results of reference toxicant testing fall within these limits. Outliers, or data which fall outside the upper and lower limits, suggest an atypical population. It is inappropriate to use that group of organisms for dredged material testing as the sensitivity of the organisms and the overall credibility of the test system would be suspect. 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). When sufficient reference toxicant data have been generated for a particular species, it may be possible to specify an acceptable LC_{50} or EC_{50} range for that species with that reference toxicant.

G.2.10.5.3 Acceptability of Test Results and Data Evaluation

For the test results to be acceptable, mean control survival must be $\geq$ 90% or the appropriate value for a particular test and end-point. If mean mortality is greater than 10% or the appropriate value for a particular test or endpoint in the control treatment for a particular test species, the test should be rejected and repeated. Unacceptable control mortality indicates that the organisms are being affected by stress other than contamination in the material being tested. Such stress 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. The potential effects of these and other variables should be carefully examined if the test is repeated.

An individual test may be conditionally acceptable if temperature, DO, and other specified conditions fall outside specifications. This depends on the degree of the departure and the objectives of the tests. The acceptability of the test will depend on the experience and professional judgment of the laboratory analyst and the reviewing staff of the regulatory authority. Any deviation from test specifications must be noted when reporting data from a test.

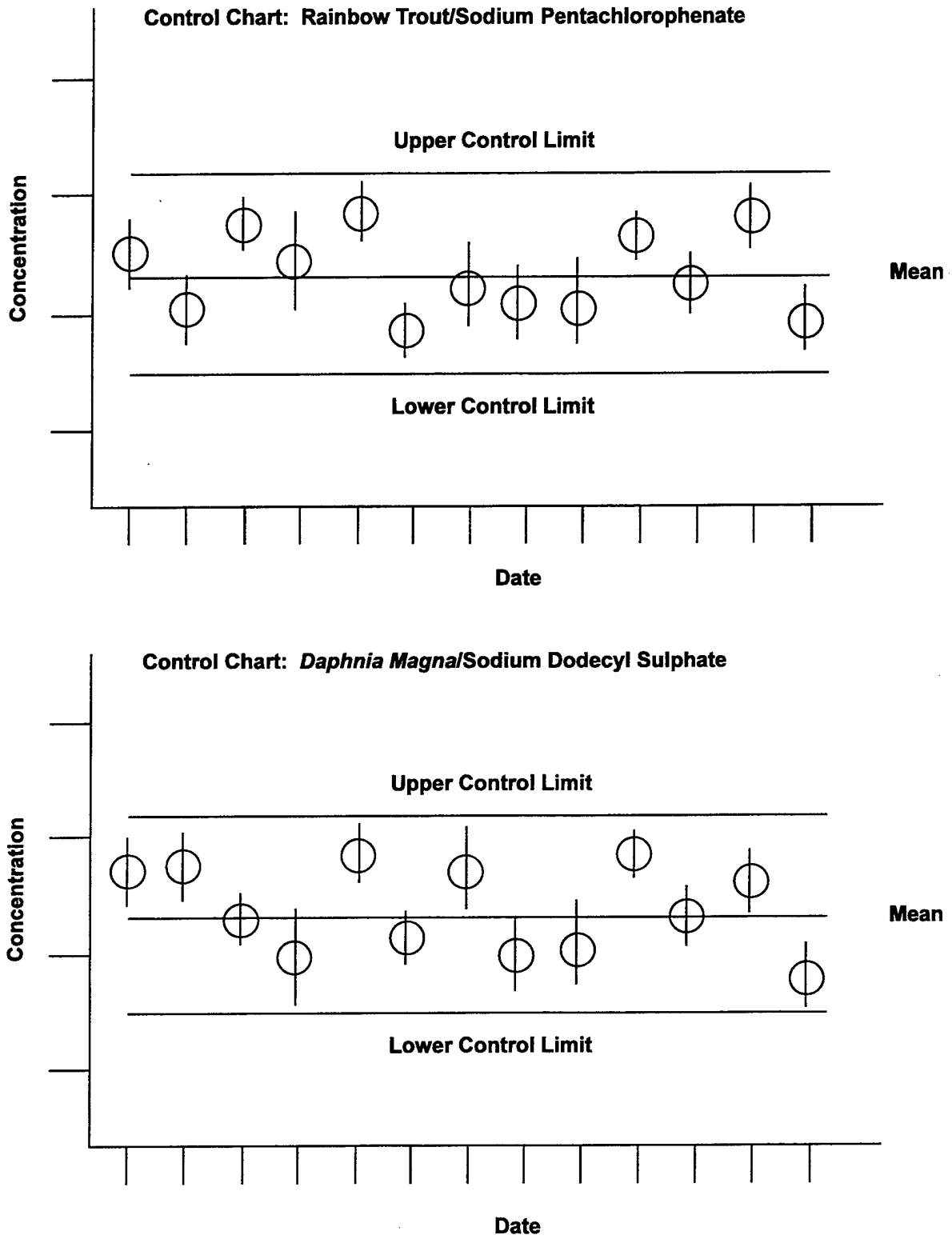


Figure G.1 Example Control Charts for Reference Toxicants.

G.2.11 Performance and System Audits

Audits include a careful evaluation of both field and laboratory QC procedures. They are an essential part of the field and laboratory QA program and consist of two basic types: performance audits and system audits. For example, analyses of performance evaluation samples may simply be used for comparison with the results of independent laboratories (a form of performance audit), or comprehensive audits may be conducted by the government of the entire field or laboratory operations (a system audit).

Performance and system audits should be conducted by individuals not directly involved in the measurement process. A performance auditor independently collects data using performance evaluation samples, field blanks, trip blanks, duplicate samples, and spiked samples. Performance audits may be conducted soon after the measurement systems begin generating data. They may be repeated periodically as required by task needs, duration, and cost. EPA (1991b) should be reviewed for auditing the performance of laboratories performing aquatic toxicity tests.

A systems audit consists of a review of the total data production process. It includes on-site reviews of field and laboratory operational systems. EPA and/or USACE will develop and conduct external system audits based on the approved project plan. An example of a systems audit checklist is provided in EPA (1995).

G.2.11.1 Pre-award Inspections

The pre-award inspection is a type of system audit for assessing the laboratory's overall capabilities. This assessment includes a determination that the laboratory personnel are appropriately qualified and that the required equipment is available and is adequately maintained. 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 government places on quality work and products. The purpose of the pre-award inspection is to verify the following:

- The laboratory has an independent QA/QC program.
- Written work plans are available for each test that describe the approach to be used in storing, handling, and analyzing samples.
- Technically sound, written standard operating procedures (SOPs) are available for all study activities.

- Qualifications and training of staff are appropriate and documented.
- Approved analytical procedures are being followed.

G.2.11.2 Interlaboratory Comparisons (Chemical Analytical Laboratories)

It is important that data collected and processed at various laboratories be comparable. As part of the performance audit process, laboratories may be required to participate in analysis of performance evaluation samples related to specific projects. In particular, laboratory proficiency testing is recommended. Laboratory proficiency must be demonstrated before a laboratory negotiates a contract and yearly thereafter. Each laboratory participating in a proficiency test is required to analyze samples prepared to a known concentration. Analytes used in preparation of the samples must originate from a recognized source of standard reference material (SRM), such as the National Institute for Standards and Technology (NIST). Proficiency testing programs already established by either EPA or the USACE may be used, or a program may be designed specifically for dredged material evaluations. Analytical results are compared with predetermined criteria of acceptability.

In addition, the performance evaluation samples prepared by EPA Environmental Monitoring and Systems Laboratory (Las Vegas, Nevada) for the Contracts Laboratory Program (CLP) may be used to assess interlaboratory comparability. Analytical results are compared with predetermined criteria of acceptability (e.g., values that fall within the 95 percent confidence interval are considered acceptable). The QA project plan should indicate, where applicable, scheduled participation in all interlaboratory calibration exercises.

Reference materials are substances with well-characterized properties that are useful for assessing the bias of an analysis and auditing analytical performances among laboratories. SRMs are certified reference materials containing precise concentrations of chemicals, accurately determined by a variety of technically valid procedures, and are issued by the National Institute of Standards and Technology. Currently, SRMs are not available for the physical measurements of all contaminants in sediments; however, where possible, available SRMs or other regional reference materials that have been repeatedly tested should be analyzed with every 20 samples processed.

SRMs for most organic compounds are not currently available for seawater, but reference materials for many inorganic chemicals may be obtained from the organizations listed in Table G.1. Seawater matrix spikes of target analytes (e.g., seawater spiked with National Institute for Standards and Technology SRM 1647 for PAH) should be used to check analytical bias. Some available SRMs for priority pollutant metals in seawater are National Research Council of Canada seawater CASS-1 and seawater NASS-2.

Table G.1 Sources of Standard Reference Materials

PCBs		
National Research Council of Canada	Marine sediment	HS-1 and HS-2
PAHs		
National Research Council of Canada	Marine sediment	HS-3, HS-4, HS-5, HS-6
National Institute for Standards and Technology	Sediment	SRM #1647 and SRM #1597
Metals		
National Bureau of Standards	Estuarine sediment	SRM #1646
National Research Council of Canada	Marine sediment	MESS-1, BCSS-1, PACS-1
	Dogfish liver	DOLT-1
	Dogfish muscle	DORM-1
	Lobster hepatopancreas	TORT-1
International Atomic Energy Agency	Marine sediment	SD-N-1/2(TM)
	Fish flesh	MA-A-2(TM)
	Mussel tissue	MAL-1(TM)

Standard reference materials (SRMs) may be obtained from the following organizations:

Organic Constituents

U.S. Department of Commerce
National Institute for Standards of Technology
Office of Standard Reference Materials
Room B3111 Chemistry Building
Gaithersburg, Maryland 20899
Telephone: (301) 975-6776

Marine Analytical Chemistry Standards Program
National Research Council of Canada
Atlantic Research Laboratory
1411 Oxford Street
Halifax, Nova Scotia, Canada B3H 3Z1
Telephone: (902) 426-8280

Inorganic Constituents

U.S. Department of Commerce
National Institute for Standards and Technology
Office of Standard Reference Materials
Room B3111 Chemistry Building
Gaithersburg, Maryland 20899
Telephone: (301) 975-6776

Marine Analytical Chemistry Standards Program
National Research Council of Canada
Division of Chemistry
Montreal Road
Ottawa, Ontario, Canada K1A 0R9
Telephone: (613) 993-2359

SRMs for organic priority pollutants in tissues are currently not available. The National Institute of Standards and Technology is presently developing SRMs for organic analytes. Tissue matrix spikes of target analytes should be used to fulfill analytical accuracy requirements for organic analyses.

Because new SRMs appear constantly, current listings of appropriate agencies should be consulted frequently. SRMs that are readily available and commonly used are included in Table G.1.

G.2.11.3 Routine Inspections

Routine system audits during the technical evaluation ensure that laboratories are complying with the QA project plan. It is suggested that checklists be developed for reviewing training records, equipment specifications, QC procedures for analytical tasks, management organization, etc. An example of a systems audit is provided in EPA (1995). Districts 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 work. Procedures for external systems audits by the Districts are similar to the internal systems audits conducted by the laboratories themselves.

G.2.12 Facilities

The QA Project Plan should provide a complete, detailed description of the physical layout of the laboratory, define space for each test area, describe traffic-flow patterns, and document special laboratory needs. The design and layout of laboratory facilities are important to maintain sample integrity and prevent cross-contamination. The specific areas to be used for the various evaluations should be identified. Aspects of the dredging study that warrant separate facilities include the following:

- receiving
- sample storage
- sample preparation
- sample testing
- reagent storage
- data reduction and analysis.

G.2.13 Preventive Maintenance

The QA project plan should describe how field and laboratory equipment essential to sample collection and analysis will be maintained in proper working order. Preventive maintenance may be in the form of: 1) scheduled maintenance activities to minimize costly downtime and ensure accuracy of measurement systems, and 2) available spare parts, backup systems, and equipment. 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.

G.2.14 Calculation of Data Quality Indicators

The calculations and equations used routinely in QA review (e.g., relative percent difference of duplicates) as well as the type of samples (e.g., blanks, replicates) analyzed to assess precision, bias, and completeness of the data must be presented in the QA project plan. Routine procedures for measuring precision and bias include use of replicate analyses, standard reference materials, and matrix spikes. Completeness can be measured for each set of data received by dividing the number of valid (i.e., accepted) measurements actually obtained by the number of measurements that were planned.

G.2.15 Corrective Actions (Management of Nonconformance Events)

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

Corrective actions fall into two categories: 1) handling of analytical or equipment malfunctions, and 2) handling of nonconformance or noncompliance with the QA requirements that have been established. During field and laboratory operations, the supervisor is responsible for correcting equipment malfunctions. All corrective measures taken must be documented and, if required, an alteration checklist must be completed.

Corrective action procedures must be described for each project and include the following elements:

- procedures for corrective actions when predetermined limits for data acceptability are exceeded (see "data quality objective" discussion in Section G.2.3)

- for each measurement system, identify the individual responsible for initiating the corrective action and also the individual responsible for approving the corrective action.

Corrective actions may be initiated as a result of other QA activities including performance audits, system audits, interlaboratory/interfield comparison studies, and QA program audits. An example of a corrective actions checklist is provided in Appendix G.4.

G.2.16 QA Reports to Management

QA Project Plans provide a mechanism for periodic reporting to management on the performance of measurement systems and data quality. At a minimum, these reports should include:

- periodic assessment of measurement data accuracy (precision and bias), and completeness

- results of performance and system audits

- significant QA problems and recommended solutions.

The individuals responsible for preparing the periodic reports should be identified. The final report for each project must include a separate QA section which summarizes data quality information contained in the periodic reports.

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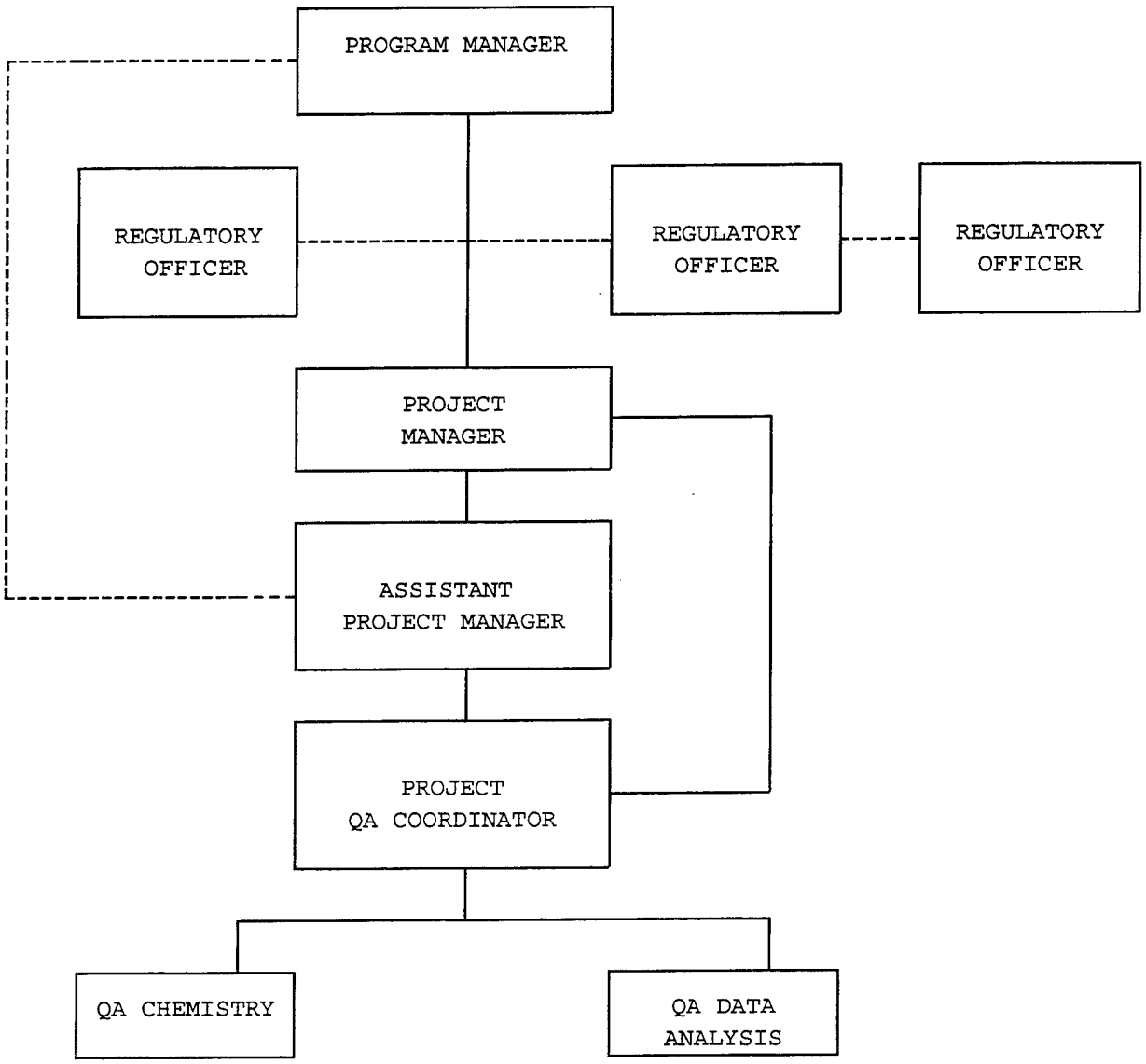
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APPENDIX G.4
EXAMPLE QA/QC CHECKLISTS, FORMS, AND RECORDS

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QA PROGRAM ORGANIZATION FLOW DIAGRAM



EXAMPLE DATA QUALITY OBJECTIVES FOR PRECISION, ACCURACY, AND COMPLETENESS

Variable	Matrix	Units	Lower Limit of Detection	Accuracy	Precision (%)	Completeness (%)	Method	Reference	Maximum Holding Time
Volatiles	Sediment	µg/kg	5	*	±30%	99%	Purge & Trap/ GC-MS	EPA abc/x-cc-yyy (1975)	10 days
Grain Size	Sediment	Percent	0.01	--	±5%	99%	Sieve & pipet		Undetermined

ALTERATION CHECKLIST

Sample Program Identification: _____

Material to be Sampled: _____

Measurement Parameter: _____

Standard Procedure for Analysis: _____

_____

Reference: _____

_____

_____

Variation from Standard Procedure: _____

_____

_____

Reason for Variation: _____

_____

_____

Resultant Change in Field Sampling Procedure: _____

_____

_____

Special Equipment, Material, or Personnel Required: _____

_____

_____

Author's Name: _____ Date: _____

Approval: _____ Title: _____

Date: _____

_____

GENERAL SAMPLE LABEL

(NAME OF SAMPLING ORGANIZATION)

PROJECT: _____

DATE: _____

TIME: _____

SAMPLE ID NO.: _____

MEDIA: _____

STATION NUMBER: _____

DEPTH: _____

PRESERVATION: _____

ANALYSES TO BE PERFORMED: _____

SAMPLED BY: _____

LAB NO.: _____

REMARKS: _____

FIELD TRACKING REPORT FORM

W/O No. _____ Page _____

FIELD TRACKING REPORT: _____-_____
(LOC-SN)

FIELD SAMPLE CODE (FSC)	BRIEF DESCRIPTION	DATE	TIME	SAMPLER

LABORATORY TRACKING REPORT FORM

W/O No. _____ Page _____

LABORATORY TRACKING REPORT: _____-_____
(LOC-SN)

FRACTION CODE	X	PREP/ANAL REQUIRED	RESPONSIBLE INDIVIDUAL	DATE DELIVERED	DATE COMPLETED

CHAIN-OF-CUSTODY RECORD

G-30

CHAIN OF CUSTODY RECORD

PROJ. NO.	PROJECT NAME					NO. OF CON-TAINERS	ANALYSIS							REMARKS
SAMPLERS: *(Signature)*														
STA. NO.	DATE	TIME	COMP	GRAB	STATION LOCATION									

Relinquished by: *(Signature)*	Date / Time	Received by: *(Signature)*	Relinquished by: *(Signature)*	Date / Time	Received by: *(Signature)*
Relinquished by: *(Signature)*	**Date / Time**	**Received by:** *(Signature)*	**Relinquished by:** *(Signature)*	**Date / Time**	**Received by:** *(Signature)*
Relinquished by: *(Signature)*	**Date / Time**	**Received for Laboratory by:** *(Signature)*	**Date / Time**	**Remarks**	

Distribution: Original Accompanies Shipment; Copy to Coordinator Field Files

STATION LOCATION LOG

DATE: _____

PROJECT: _____

STATION LOCATION: _____

DESCRIPTION OF SAMPLES COLLECTED: _____

SPC ZONE: _____(N/S) EAST: _____ NORTH: _____

_____

LOCATION:

Bottom Depth: _____ (ft) _____ (m) Tide: ± _____ (m) MLLW: _____ (ft) _____ (m)

LORAN C: LOP1 _____ LOP2 _____

Variable Radar Range: _____

_____

_____

Visual Fixes: (Note: Please tape any drawings to back of this sheet)

_____

_____

_____

Photos - Roll: _____ Pictures: _____

PID Reading (range): _____

Comments: _____

_____

_____

RECORDER: _____ SIGNATURE: _____ ORG. CORE ____ DATE: _____

_____