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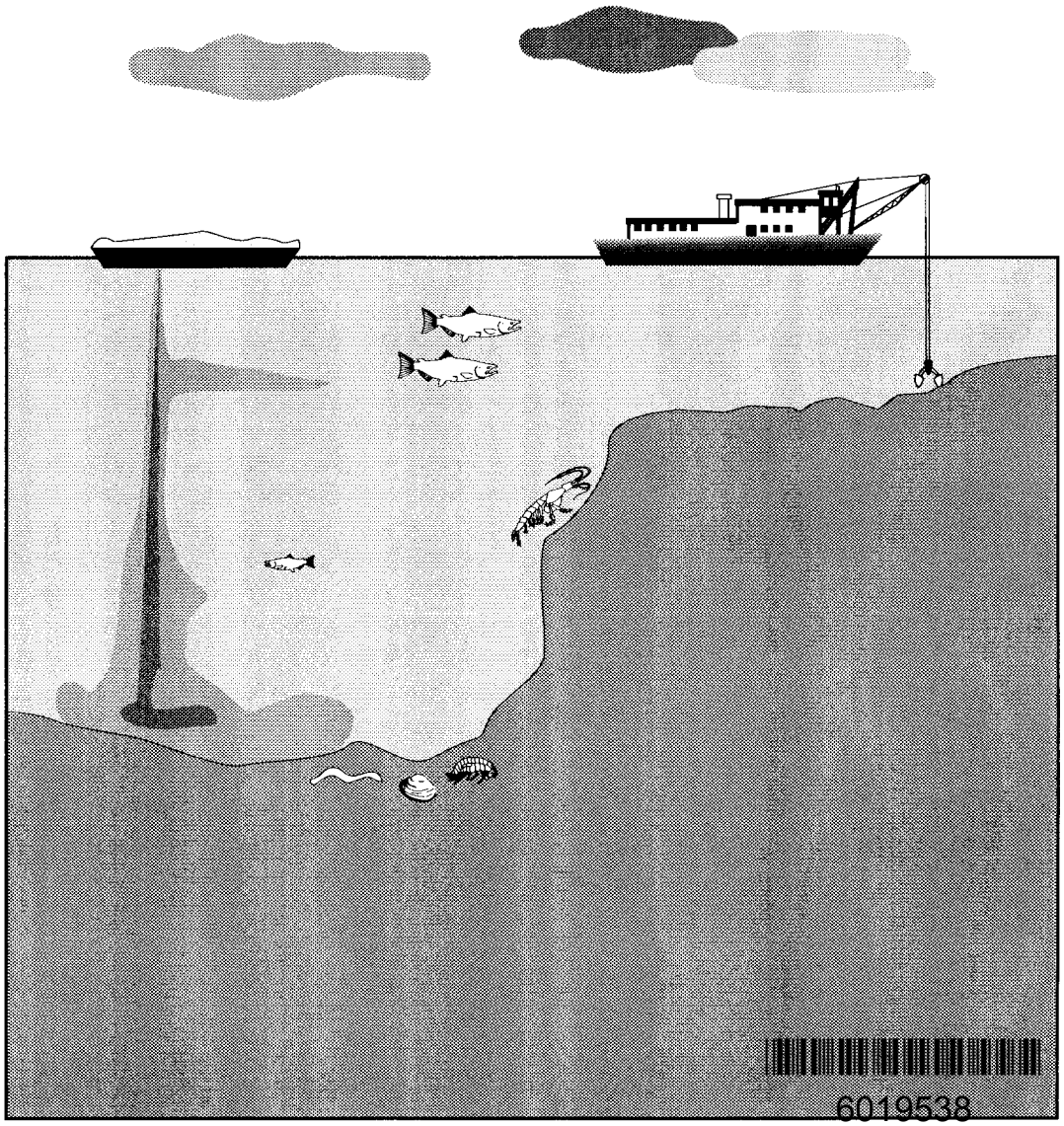
# Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. - Testing Manual

## Inland Testing Manual



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

## (INLAND TESTING MANUAL)

**Prepared by**

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**and**

**DEPARTMENT OF THE ARMY**
**United States Army Corps of Engineers**
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**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 | 1$yd^3$ = 27$ft^3$ = 764.555 L = 0.7646$m^3$ |
| 1mL = 1000μL | 1 acre-ft = 1233.482$m^3$ |
| 1cc = $10^{-6}m^3$ | 1 gallon (US) = 3785cc |
| | 1 $ft^3$ = 0.0283$m^3$= 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 = 0.45$ft^3$/s |
| | $yd^3$/s = 3.366 gal/s = 12.743 L/s |
| **AREA:** | |
| 1 $m^2$ = 10.764$ft^2$ | 1 $ft^2$ = 0.0929$m^2$ |
| 1 hectare (ha) = 10000$m^2$ = 2.471 acres | 1 acre = 4046.856$m^2$ = 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

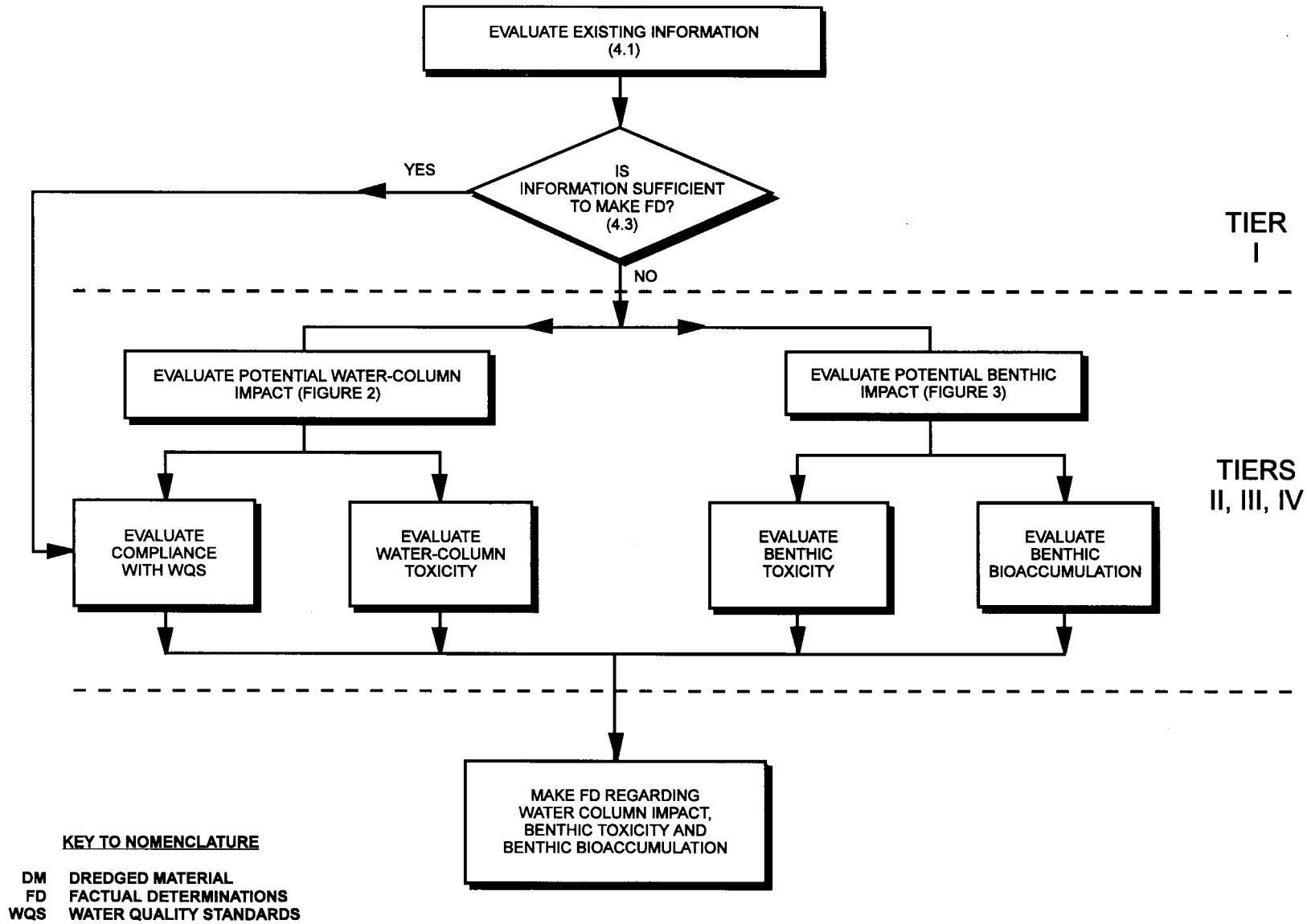



Figure 3-1. Simplified Overview of Tiered Approach to Evaluating Potential Impact of Aquatic Disposal of Dredged Material.

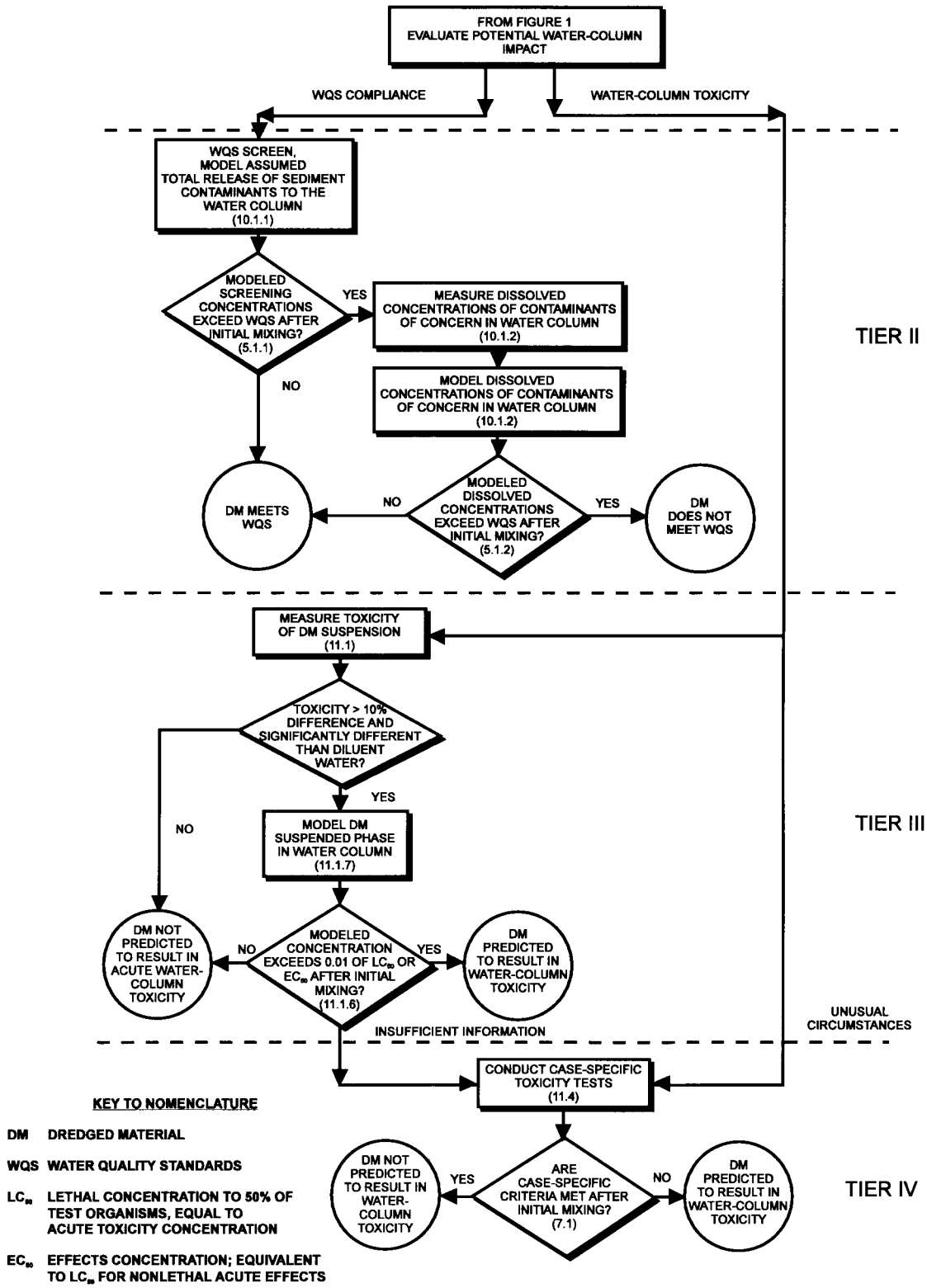



Figure 3-2. Illustration of Tiered Approach to Evaluating Potential Water Column Impacts of Dredged Material.

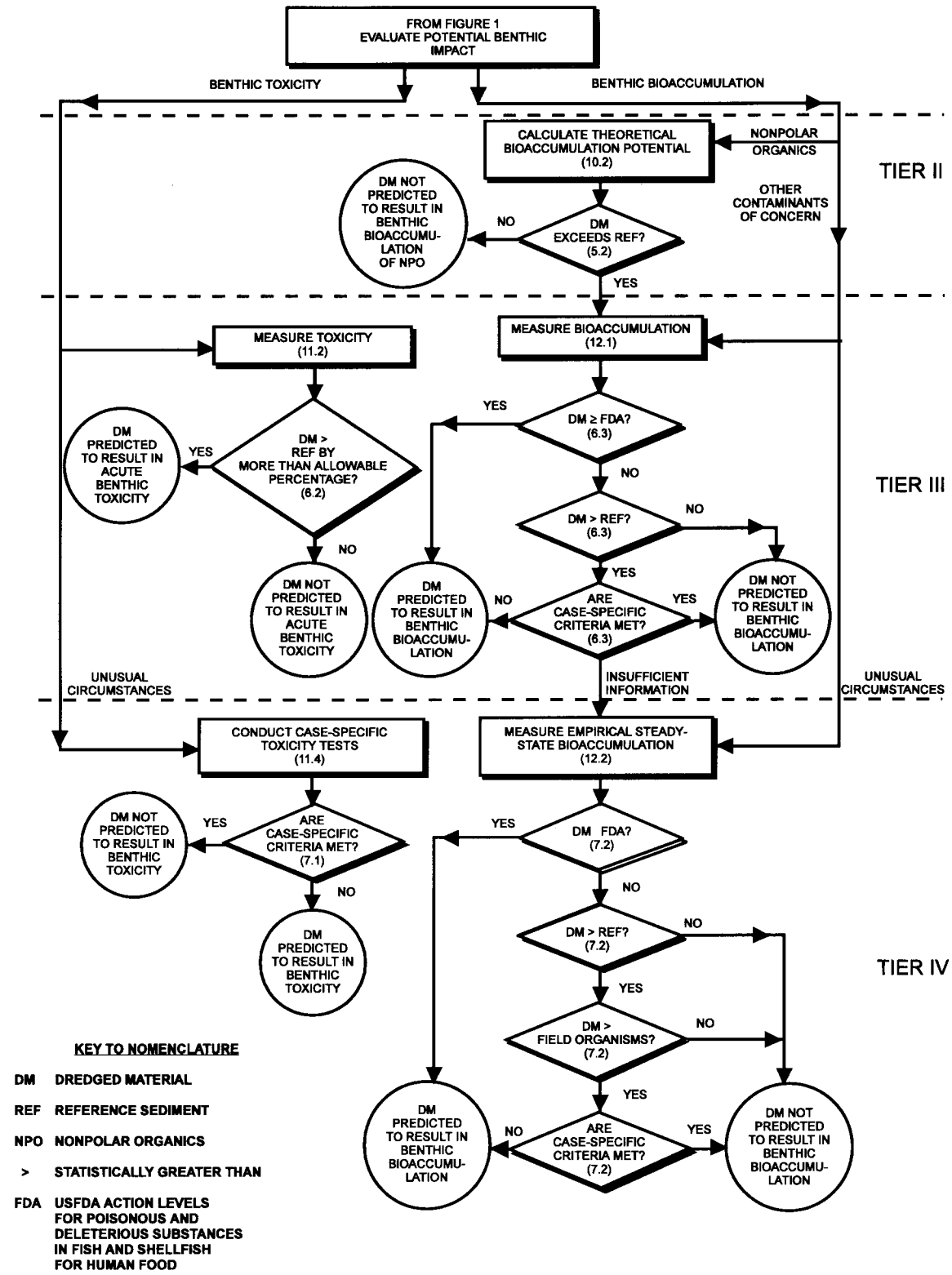



Figure 3-3. Illustration of Tiered Approach to Evaluating Potential Benthic Impacts of Deposited Dredged Material.

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.

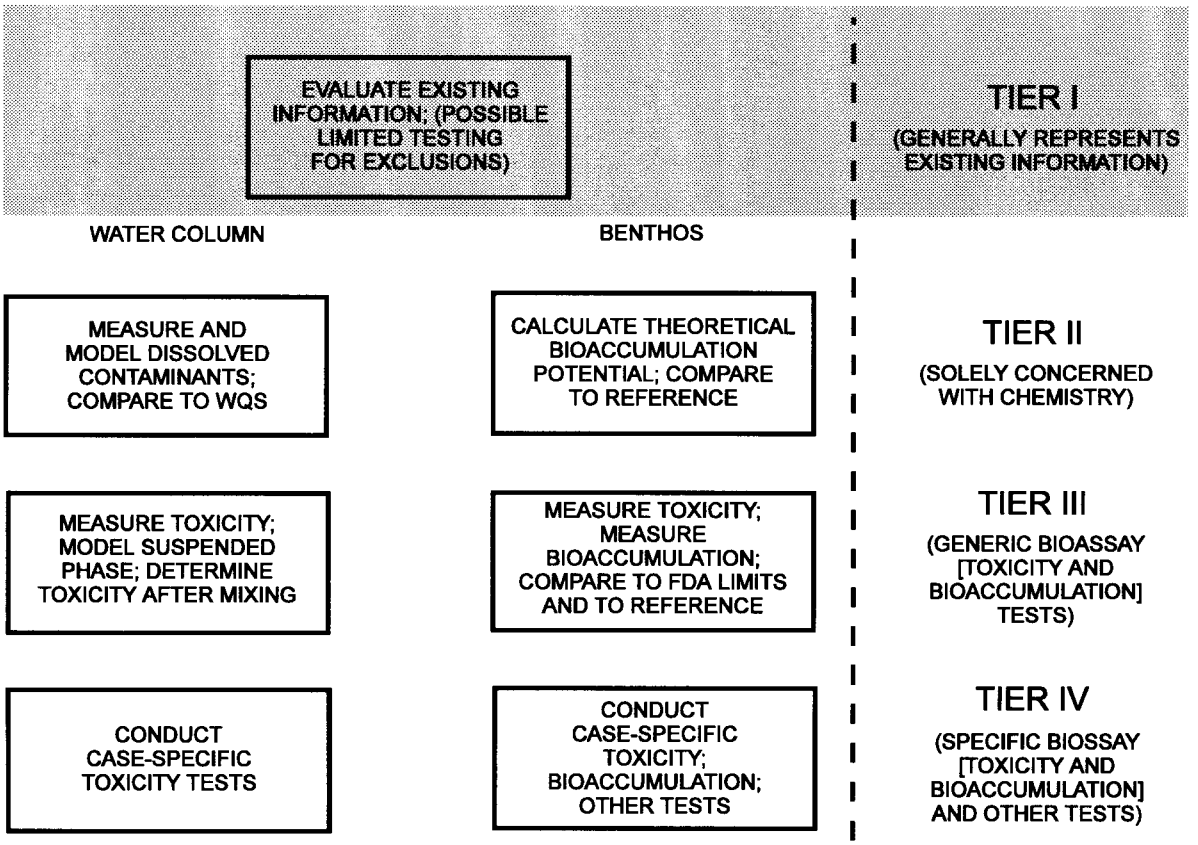
Appendix D, Statistical Methods, provides guidance for conducting statistical comparisons for the reference point and reference area approaches. It does not provide guidance for the use of either of the "periodic" approaches.

### 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 | AntiFouling 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

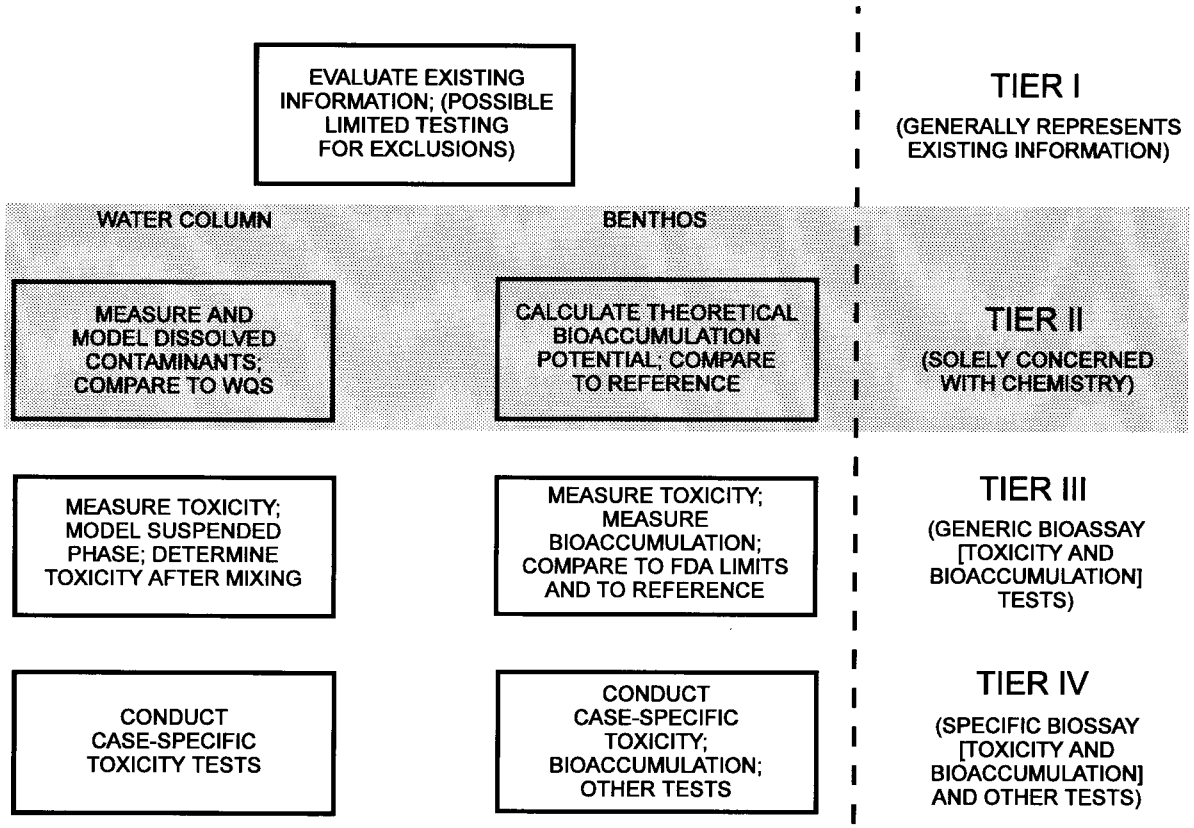
After consideration of all available information, one of the following conclusions is reached (Figure 3-1):

- 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:

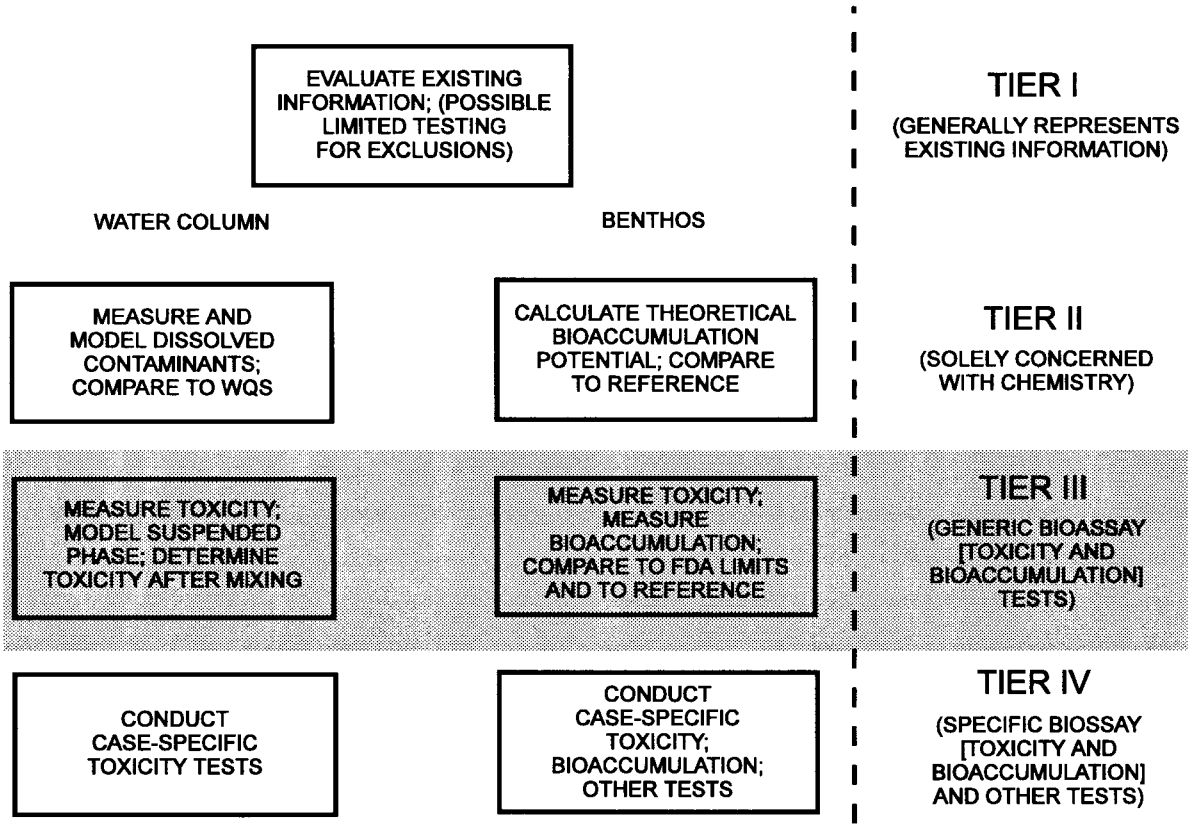
- The available WQS requirements are met. Further information on water column toxicity must be evaluated in Tier III when there are contaminants of concern for which applicable WQS are not available or where interactive effects are of concern.

- 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, <u>and</u>

- 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

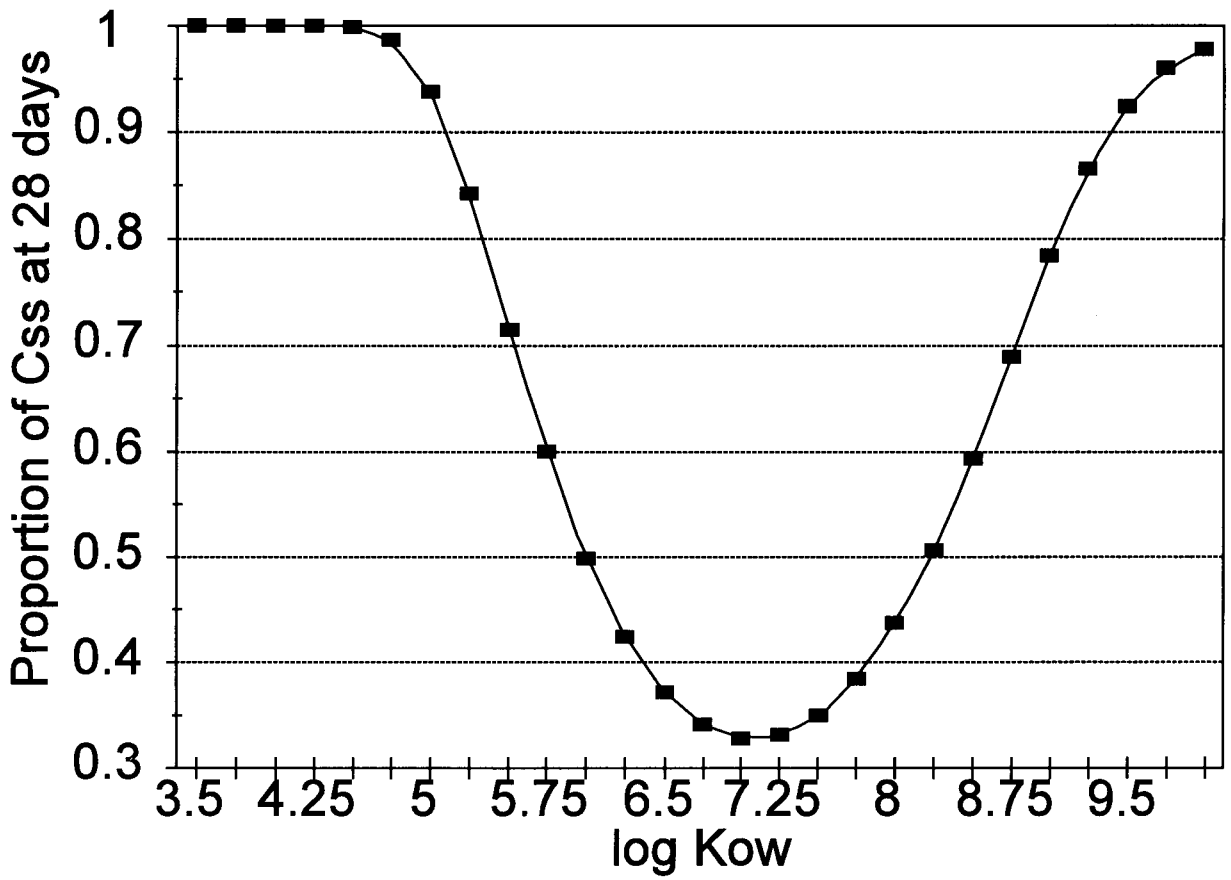



Figure 6-1. Expected proportion of steady-state concentration (Css) of neutral organic compounds reached in 28-day laboratory exposures. The proportion is a function of the log Kow of the compound of interest. Consult Section 9.5.1 (Table 9-5) for appropriate log Kow values. Figure adapted from McFarland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

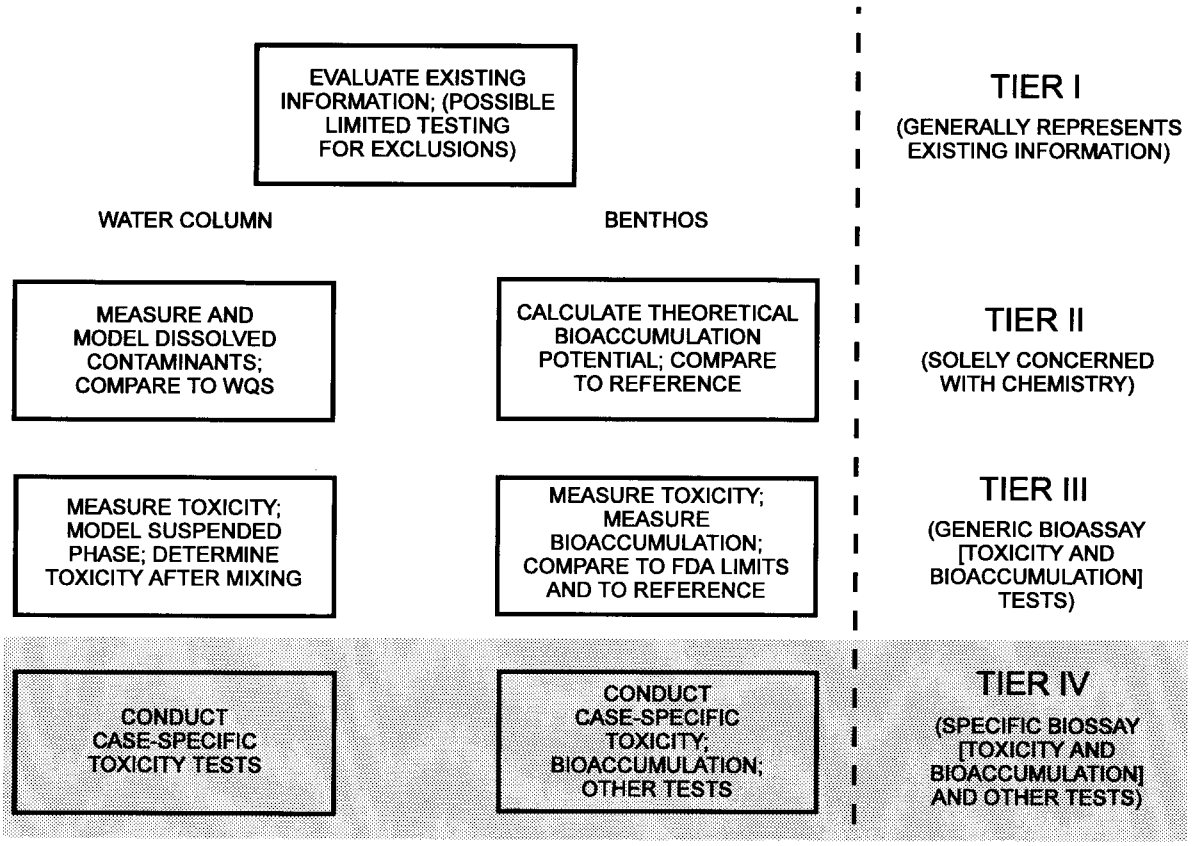
## 6.4 Tier III Conclusions

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.

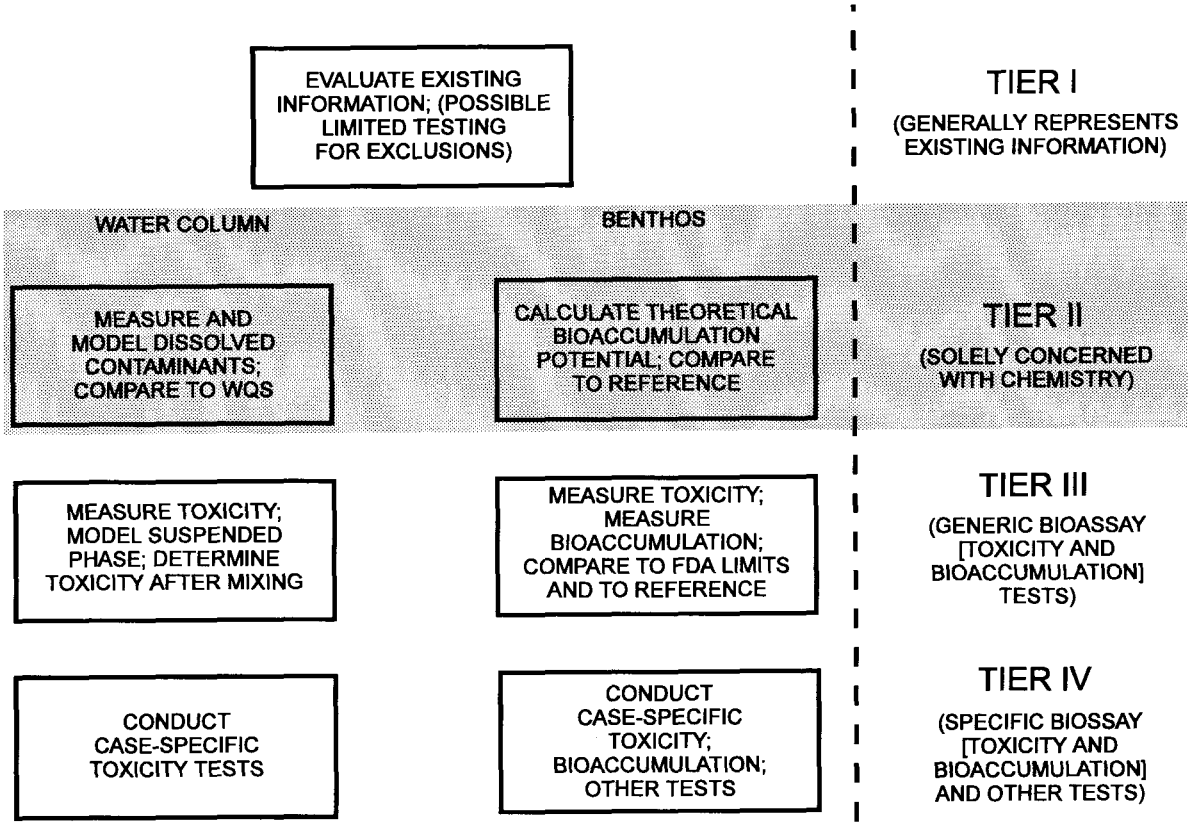
Existing methods for priority pollutant tissue analysis involve two separate procedures: one for organic compounds and another for metals. The recommended methods for the analysis of semivolatile organic pollutants are described in NOAA (1989). The procedure involves serial extraction of homogenized 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

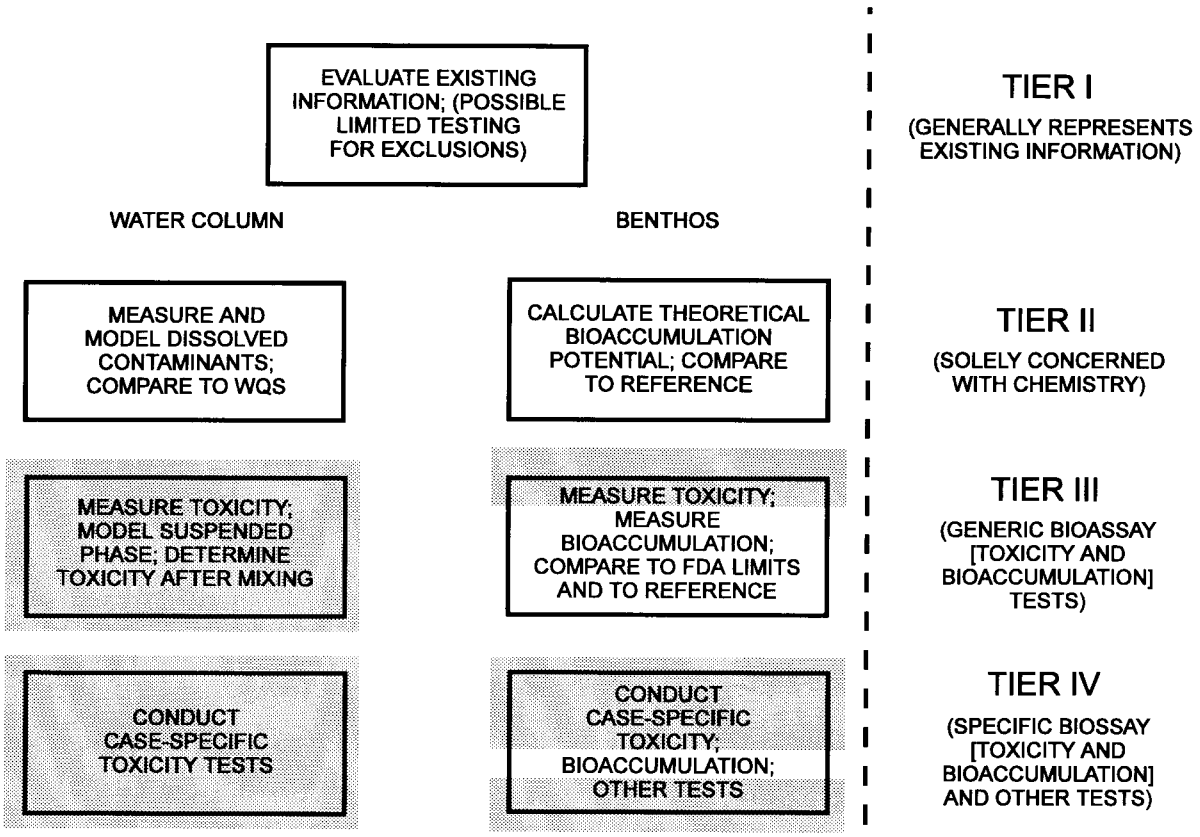
$C_s$ = concentration of nonpolar organic chemical in the dredged material or reference sediment (any units of concentration may be used);

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.

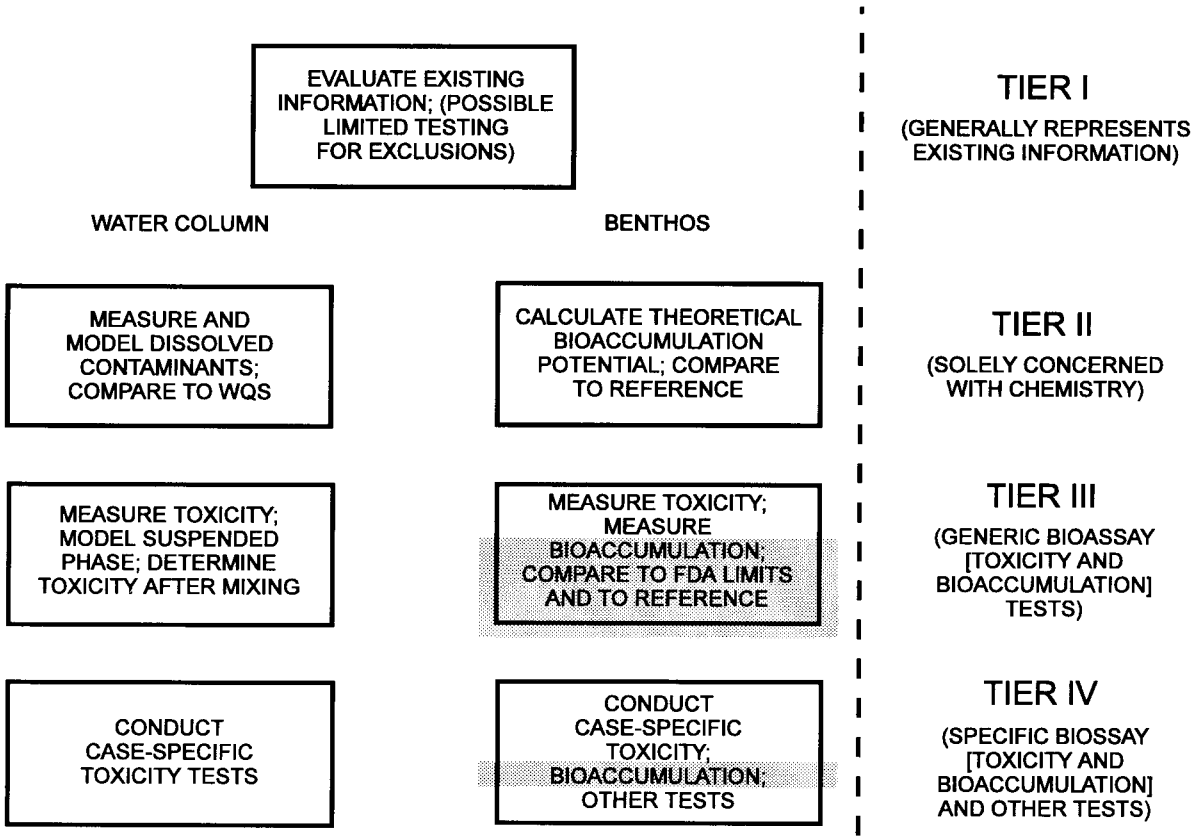
## 11.3 Tier IV: Chronic/Sublethal Effects Evaluations

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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