EPA
Region 1, New England

U.S. Army Corps of
Engineers
New England District

DRAFT

# REGIONAL IMPLEMENTATION MANUAL

for the

# EVALUATION OF DREDGED MATERIAL PROPOSED FOR DISPOSAL IN NEW ENGLAND WATERS

Prepared by

**U.S. EPA - NEW ENGLAND**

and the

**U.S. ARMY CORPS OF ENGINEERS,
NEW ENGLAND DISTRICT**

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# List of Abreviations

| | |
|---|---|
| AED | Atlantic Ecology Division, EPA Research Lab, Narragansett, Rhode Island |
| APHA | American Public Health Association |
| ASTM | American Society of Standards and Materials |
| CRM | Certified Reference Material |
| CWA | Clean Water Act |
| DM | Dredged Material |
| DOA | Department of the Army |
| ENG | U.S. Army Engineering Form |
| EPA | U.S. Environmental Protection Agency |
| FWS | U.S. Fish and Wildlife Service |
| GC/MS | Gas Chromatography/Mass Spectroscopy |
| ITM | Inland Testing Manual |
| LC50 | Median Lethal Concentration |
| LPC | Limiting Permissible Concentration |
| LIS | Long Island Sound |
| LQAP | Laboratory Quality Assurance Plan |
| MAS | Marine Analysis Section, New England District, Corps of Engineers |
| MDL | Method Detection Limit |
| MLLW | Mean Lower Low Water |
| MLW | Mean Low Water |
| MPRSA | Marine Protection, Research and Sanctuaries Act |
| NAE | New England District, U.S. Army Corps of Engineers |
| NMFS | National Marine Fisheries Service |
| NOAA | National Oceanic and Atmospheric Administration |
| NYDEC | New York Department of Environmental Conservation |
| PAH | Polycyclic Aromatic Hydrocarbon |
| PCB | Polychlorinated Biphenyl |
| PQL | Practical Quantitation Limit |
| PSEP | Puget Sound Estuary Program |
| ppb | parts per billion |
| ppm | parts per million |
| pptr. | parts per trillion |
| QA/QC | Quality Assurance/Quality Control |
| RIM | Regional Implementation Manual |
| RL | Reporting Limit |
| SAP | Sampling and Analysis Plan |
| SIM | Selected Ion Monitoring |
| SRM | Standard Reference Material |
| TBP | Theoretical Bioaccumulation Potential |
| TOC | Total Organic carbon |
| TQL | Target Quantitation Limit |
| USACE | U.S. Army Corps of Engineers |
| WQC | Water Quality Criteria |

# 1. INTRODUCTION

This Regional Implementation Manual (RIM) presents sediment testing guidelines and reporting requirements for applicants who wish to obtain a Department of Army permit from the New England District (NAE) of the U.S. Army Corps of Engineers (Corps) for dredging and the open water disposal of dredged material projects as well as federal navigation projects. This guidance is consistent with national guidance (described below) and has been approved by the U.S. Environmental Protection Agency (EPA) and the Corps in cooperation with the U.S. Fish and Wildlife Service (FWS), National Marine Fisheries Service (NMFS) and the various permitting and environmental resource agencies of the five coastal New England states: Maine, New Hampshire, Massachusetts, Rhode Island and Connecticut.

This manual implements the national testing guidelines under Section 103 of the Marine Protection, Research and Sanctuaries Act (MPRSA) (33 USC 1401 et seq.) and Section 404 of the Clean Water Act (CWA) (33 USC 1344 et seq.). The MPRSA governs (1) all disposal projects in New England ocean waters (seaward of the territorial sea baseline), and (2) all Federal disposal projects of any amount, and those non-Federal disposal projects exceeding 25,000 cubic yards in size, in Long Island Sound. In addition, Section 404 of the Clean Water Act regulates the disposal of dredged and fill materials into waters of the U.S. landward of the territorial sea baseline and fill material within the territorial sea. The guidance and requirements specified in this document will be used by the regulatory agencies for all disposal activities subject to both Section 103 of the MPRSA (40 CFR Parts 227.6 and 227.13) and Section 404 of the Clean Water Act (40 CFR Parts 230.60 and 61).

The MPRSA requires that operations involving the transportation and discharge of dredged materials in ocean waters are to be evaluated to determine the potential impact to the marine environment. The proposed disposal must be evaluated through the use of criteria published by the EPA in Title 40 of the Code of Federal Regulations, Parts 220-228 (40 CFR 220-228). In accordance with Subsection 227.27 (b) of the regulations, EPA and Corps developed a testing manual to define procedures for evaluating the suitability of dredged material for ocean disposal that are based upon the testing requirements in the regulations. National guidance is provided in the document "Evaluation of Dredged Material Proposed for Ocean Disposal Testing Manual" commonly known and hereafter referred to as the "Green Book" (EPA/USACE, 1991). This document replaces the first testing manual "Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters" (EPA/USACE, 1977).

Under the CWA, the testing described here will assist the Corps of Engineers in making the factual determinations regarding the effect of the discharge on the aquatic ecosystem and compliance with the 404(b)(1) guidelines (40 CFR Parts 230.10 and 230.11). It implements the manual "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. - Testing Manual (hereafter known as the "Inland Testing Manual" (or ITM)) (EPA/USACE 1998) as specified in 40 CFR Parts 230.60 and 230.61.

The 1991 Green Book and 1998 ITM provide new and improved testing methods and contain revised guidance that reflects the regulatory experience gained since the 1977 testing manual was published. The Green Book, ITM and the companion quality assurance/quality control (QA/QC) manual (EPA/USACE 1995) provide guidance on the tiered-testing approach, sampling methodology, testing procedures, statistical methods and QA/QC. The purpose of the national guidance is to provide a framework to insure consistency with the national ocean disposal regulatory program.

This Regional Implementation Manual (RIM) of the 1991 Green Book and 1998 ITM applies the national guidance to the New England area, providing additional guidance agreed upon by EPA Region I and NAE in cooperation with the above listed agencies. It replaces the previous regional manual entitled "Guidance for Performing Tests on Dredged Material to be Disposed of in Open Waters" (EPA/NAE, 1989) which implemented the 1977 national guidance document. This current manual provides needed supplementary guidance on: permit application requirements, data and reporting requirements, a list of the contaminants of concern, species for biological testing and specific procedural requirements agreed upon by state and federal agencies. This will avoid unnecessary confusion and possible delays or expenses through the submission of improper data. The reader should be aware that this document does not attempt to duplicate or replace the detailed information contained in the Green Book, ITM or the QA/QC manual except where noted. However, this document is designed to be used in conjunction with these manuals providing additional information or clarification when needed. Specific references to appropriate sections are provided.

New and more advanced testing procedures and guidelines are continually being developed and refined by the research and development laboratories of the EPA and USACE. In addition, ongoing monitoring of designated disposal sites in New England waters under the NAE Disposal Area Monitoring System (DAMOS) program can provide effects-based feedback to the regulatory agencies allowing them to make more refined, environmentally sensitive and efficient decisions regarding the acceptability of open water disposal of dredged material. It can also provide the necessary information on whether any site-specific criteria may be needed for a particular disposal site. As a result, this document will be revised as needed to incorporate any necessary modifications of the testing guidance.

All application information, as discussed in the following sections, should be submitted by applicants to the NAE Corps of Engineers office in Concord, Massachusetts. The Corps will supply copies of the information to the other Federal agencies including EPA, FWS and NMFS. Note that applicants are required to contact the appropriate state regulatory agency directly. The applicant should know that additional information may be required on a project by project basis.

Questions about this manual should be directed to:

U.S. Army Corps of Engineers
New England District
Regulatory Division
696 Virginia Road
Concord, MA 01742-2751
(978) 318-8335

or

U.S. Environmental Protection Agency
Region I - New England
Office of Ecosystem Protection (CWQ)
One Congress Street, Suite 1100
Boston, MA 02114-2023
(617) 918-1553

# 2.0 ADMINISTRATIVE REQUIREMENTS

## 2.1 General

When applying for a Department of the Army permit to dispose of dredged material into open water, the applicant will be required by the NAE's Marine Analysis Section to provide the information indicated below. This information represents the first of four information tiers used in the evaluation of dredged material. As discussed in Section 3, it is possible that the evaluative processes in the remaining tiers (including biological testing of the materials proposed for dredging) would be necessary.

Additional guidance on preparing applications can be found in the most recent edition of NAE's GUIDE FOR PERMIT APPLICANTS Useful general application information, application forms, and sample project plans are available in the book (also available on the NAE website at www.nae.usace.army.mil). Contact the Regulatory Reception Center to ensure you have the most current copy, phone number 978-318-8338.

Upon receipt of a permit application or pre-application inquiry, NAE will assign a Regulatory Division Permits Project Manager who will serve as the applicant's point of contact throughout the review process. Information required for review by the Corps and coordinating Federal and State agencies include, but are not limited to the following data.

1. A statement describing why the proposed dredging is required, if it is "new, or improvement" or "maintenance" dredging, and the area (square feet) and volume (cubic yards) of material to be dredged. If the project is comprised of several "segments"(e.g., marina basin and an entrance channel), volumes and square footage information should be provided separately for each. The volume estimate(s) should include the maximum estimate overdepth. Current and proposed water depths should be described based on mean low water.

2. Alternative disposal locations with information in sufficient detail to evaluate their potential for use. This would include a comprehensive survey of potential upland, beneficial use and aquatic sites and information utilized in their evaluation.

3. The date when the project was last dredged and any previous sediment and biological effects test data for this or nearby projects which would aid in typifying project sediments. In the absence of any previous test data, a description of the bottom material should be provided (e.g., rock, sand, vegetated, etc.).

4. Information on locations of outfalls, non-point sources of contaminants and any recent contaminant spills must also be included as described in Chapters 2 and 8 of the ITM (EPA/ACE 1998). These data may be obtained from sources such as State water pollution control agencies (e.g., Department of Environmental Protection), U.S. Coast Guard and harbormasters. The sources of all information must be properly documented.

5. Two legible copies of 8.5" X 11.0" drawings (Figure 1) including plan views and cross sections of the area to be dredged with the following information noted:

-Depth of dredging referenced to MLW (mean low water) or MLLW ()
-Depth of proposed overdredge indicated
-Existing depths referenced to MLW or MLLW
-Square footage of the area to be dredged
-Outfall locations (industrial discharges, etc.)
-Non-point sources of contaminants (parking lots, oil storage tanks, hazardous waste, etc.)
-Proposed and historical sampling locations (if appropriate)
-Readily identifiable landmarks and a project locus insert

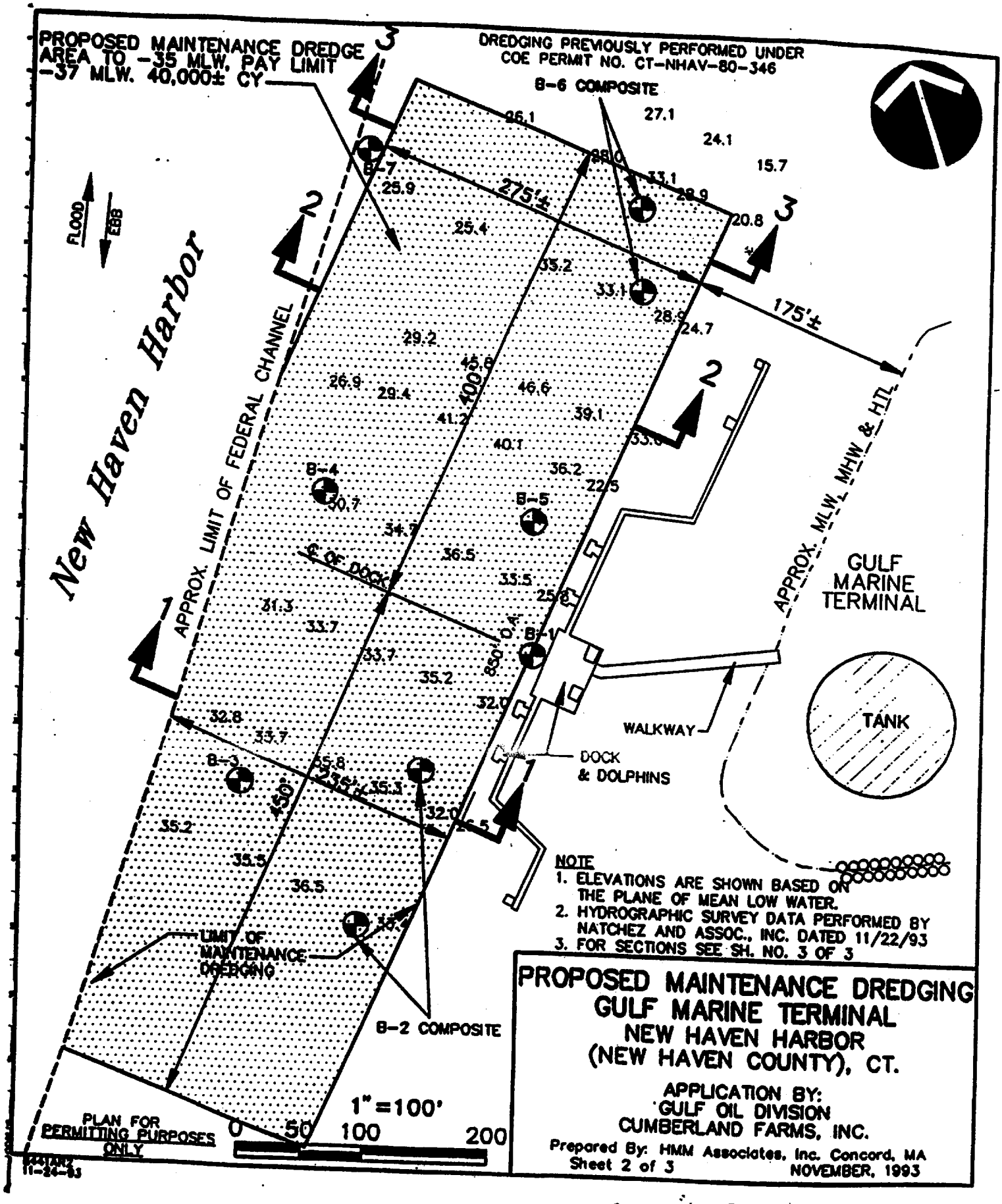
6. Type of dredging equipment to be used (clamshell, hydraulic, etc.) and any unique handling procedures to be used, such as a sealed clamshell or special runback controls.

7. Proposed dredged material disposal site. A locus sheet and detailed plan view should be provided for each upland disposal site. The plans must include information on any significant resources near the proposed site, limits of regulated areas if applicable (e.g., wetlands and waterbodies), and for open water sites, whether the site is a containment (i.e., depositional) or dispersive site.

If beach nourishment is proposed, plans should include high tide line, mean high water, and mean low water, as well as a delineation of any vegetated areas and/or resource areas in the vicinity (e.g., shellfish beds). Grain size information of the beach sediment will likely be required for beach nourishment proposals, for comparison to the dredged material, to insure they are compatible.

8. Dewatering site. If the material is to be dewatered, the following information should be provided: a description of the site; a location map; plan view and cross section of the dewatering site; calculations used to determine the capacity of the dewatering area; and details of the methods to be used to control runback.

FIGURE 1. Example Drawing of Area Proposed for Dredging



## 2.2 Coordination

Early coordination with the NAE Regulatory staff (obtained through the Regulatory Project Manager) is required to determine the sediment contaminant analyses needed, and for development and approval of a project Sampling and Analysis Plan (SAP), including the proper techniques, location and number of samples to be taken (See Chapter 4).

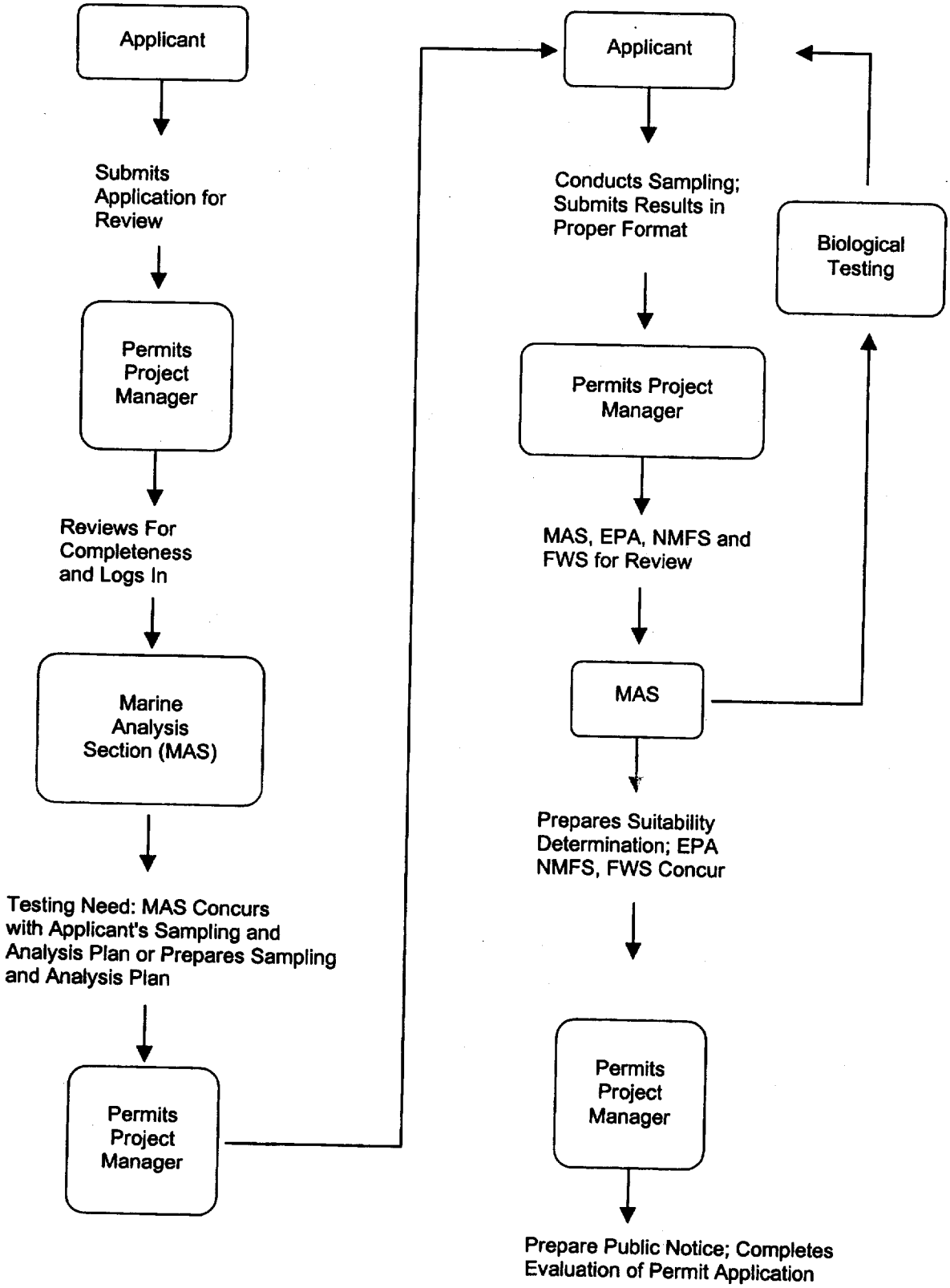
Prior to any sampling and testing, the applicant must ensure submission to NAE of the following:

1) A laboratory quality assurance plan (LQAP) providing standard quality assurance/quality control procedures used by the contractor laboratory (unless previously submitted) (Note: NAE and EPA are currently programmatically reviewing LQAPs from laboratories performing testing for this regulatory program. A 24 month grace period will be allowed for existing labs to submit LQAPs from the effective date of this document. After that date, any new labs will be required to submit and have approved LQAPs before any project data can be submitted.); and

2) A project Sampling and Analysis Plan (SAP) providing supplemental project-specific information on the actual field sampling effort and associated quality assurance measures (See Chapter 4).

The Federal permitting process (Figure 2) involves a comprehensive evaluative process and requires a multi-agency review of dredged material suitability decisions. Of prime importance is Region I, EPA which has the authority to review, approve, or propose conditions upon permits for open water disposal, and the National Marine Fisheries Service which reviews project evaluative steps and provides information on endangered species and Essential Fish Habitat and other biological resources. Early coordination (application or pre-application) ensures that unnecessary delays do not become a factor in the review process.

Figure 2. Generalized Coordination Procedure for Sediment Suitability Determination



# 3.0 TIERED TESTING

The tiered approach to testing provides increasing levels of investigative intensity to generate the information necessary to evaluate the proposed disposal of dredged material at an open water site. It provides for optimal use of resources by focusing the least effort on dredging operations where the potential (or lack thereof) for unacceptable adverse impact is clear, and expending the most effort on operations to determine the potential for impact. This approach is described in detail in Chapters 4-7 of the 1991 Green Book and Chapters 3-7 of the ITM. These chapters should be read thoroughly in either manual, depending upon the jurisdiction, to ensure a full understanding of all tiered testing requirements. A brief description of the tiered testing approach is presented below and illustrated in Figure 3. Prior to undertaking any testing, applicants must coordinate with their Corps Project Manager.

## 3.1. *Tier I* - Review of Existing Information and Identification of Contaminants of Concern.

The purpose of Tier I evaluations is to determine if existing information on proposed dredged material is sufficient to show compliance with regulations and to determine contaminants of concern. A comprehensive review of existing and readily available information is required to make this determination. If existing test data are considered inadequate to evaluate the proposed project, new sediment chemical and biological testing is required.

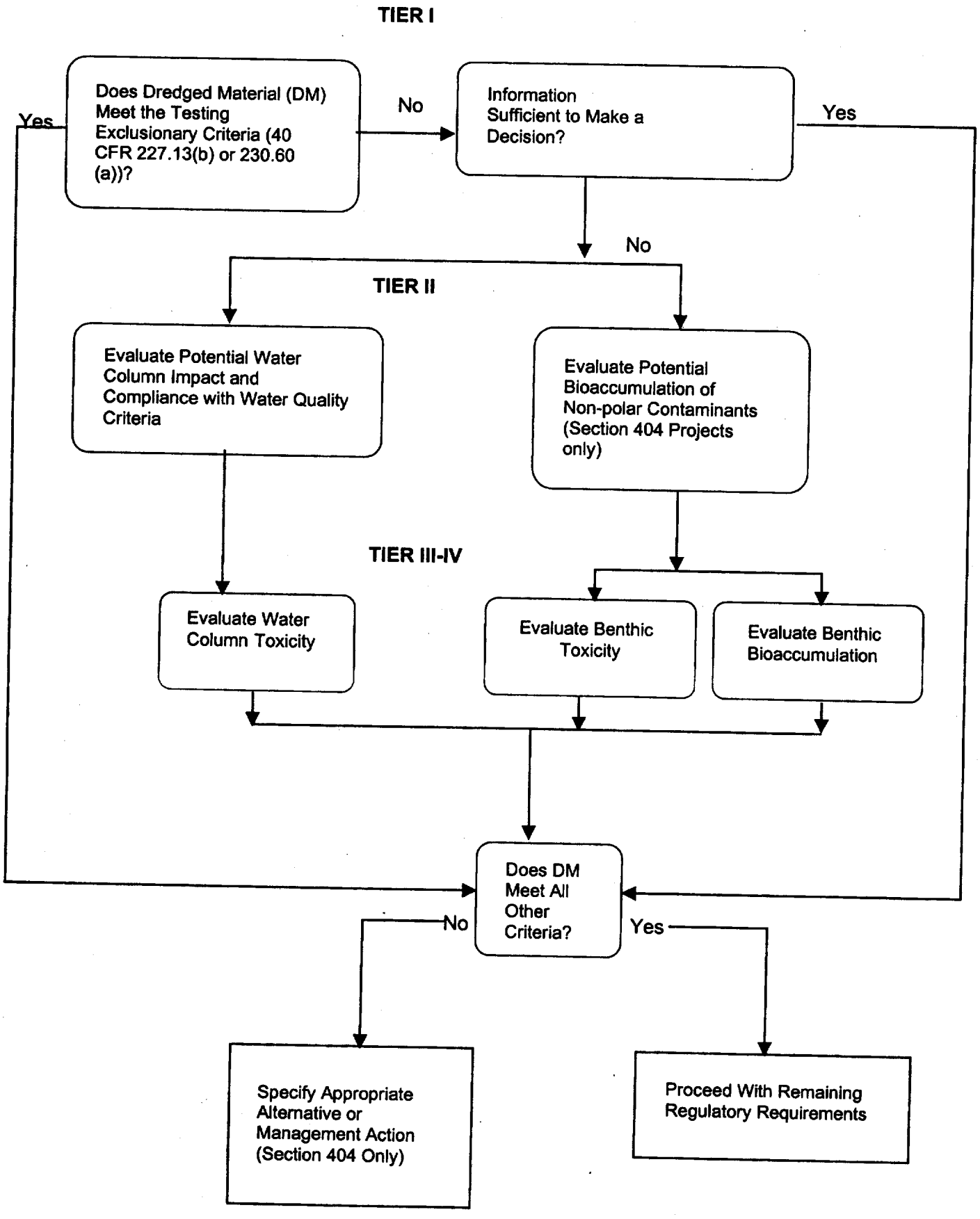
## 3.2. *Tier II* - Water Column and Potential Bioaccumulation Analyses.

Tier II consists of evaluation of water quality criteria (WQC) compliance using a numerical based mixing model (Appendix B, Green Book; Appendix C, ITM) and an evaluation for potential bioaccumulation using calculations of Theoretical Bioaccumulation Potential (Section 5.2 of the ITM) for non-polar contaminants of concern. At this time, the TBP is not used for projects subject to MPRSA (Green Book).

## 3.3. *Tier III* - Toxicity and Bioaccumulation Testing.

Tier III testing is used to provide data that allows an impact assessment of the contaminants of concern through use of toxicity and bioaccumulation tests with appropriate, sensitive organisms (see Tables 6 and 7 for test organisms). Both water column toxicity testing and benthic toxicity testing are required. Bioaccumulation testing is used to determine the potential for uptake of sediment contaminants at the disposal site by benthic organisms.

Figure 3. Generalized Tiered Process for Review of Dredging Projects



3.4. **Tier IV** - Long Term Bioassays and Bioaccumulation Tests, Risk Evaluations and other case-specific testing/evaluations.

Under unusual circumstances, such as when a unique resource or resource area is involved, it may be necessary to evaluate long-term effects of proposed dredged material on appropriate sensitive aquatic organisms and human health risks. A risk assessment, prepared by EPA may be required to interpret bioaccumulation results. Because of the limited availability of appropriate and widely accepted procedures, each test is selected to address specific concerns of each disposal operation (Section 7.1 Green Book; and Section 7 of the ITM). In a situation such as described, extremely close coordination with Region I, EPA and NAE in all aspects of Tier IV testing is required.

# 4.0. SAMPLING METHODOLOGY

The importance of a well-designed sampling program is underscored by the fact that an evaluation of the potential impacts of a proposed dredging project is only as complete and reliable as the sampling upon which it is based. The quality of information gathered through the tiered testing process is impacted by the following sampling related factors: a) collecting representative samples; b) using appropriate sampling techniques; and c) protecting or preserving the samples until they are tested. It is the responsibility of the applicant to ensure that samples taken for a proposed project meet the Quality Assurance/Quality Control (QA/QC) requirements presented below and discussed in Chapter 8 of the Green Book (EPA/USACE 1991), Chapter 8 of the Inland Testing Manual (ITM) (EPA/USACE 1998), and the Quality Assurance manual (EPA/USACE 1995). **Failure to meet these requirements or follow any specified procedure without NAE approval will likely cause rejection of the testing results.**

Within 24 months of the effective date of this manual, each Laboratory must have an approved Laboratory Quality Assurance Plan (LQAP) (see Chapter 2) on file with the Corps in order for its sampling and test data and analysis to be accepted for permit applications or for proposed Corps federal dredging and disposal projects. As mentioned in Section 2, after that date, any new labs will be required to submit and have approved LQAPs before any project data can be submitted.

### 4.1 Development of a Project Sampling and Analysis Plan (SAP)

In addition to the LQAP, applicants must have a project Sampling and Analysis Plan (SAP) (see Chapter 2) which together make up the Quality Assurance Project Plan. Applicants may submit a proposed sampling plan for approval to NAE, or request a SAP be prepared by NAE based on submittal of project plans. The plan must be coordinated with the appropriate state agencies. NAE will develop and/or approve the SAP in coordination with the federal agencies (and state agencies if appropriate). NAE will provide the approved SAP to the applicant, including the number and location of samples, the required analytes, target quantitation limits (TQL) (see below) and other project specific information supplemental to the LQAP. The approved SAP must be implemented by the applicant. Any changes to the approved SAP must be submitted to and accepted by NAE and be approved in writing prior to sampling.

**Please note that applicants should not, under any circumstances, undertake field sampling and analysis without first coordinating with the Corps and receiving an approved SAP from the Corps.**

The following data must be included with the proposed project SAP:

- a brief project description, contract lab name/address and verification that the LQAP is on file with NAE;
- reference/disposal site location (see below);
- station-specific sampling procedures (including sampling gear and proposed positioning methodology) and description;
- sample handling/storage procedures; and
- analytical procedures and detection levels (see Chapter 5).

Following approval of the SAP and concurrent with submission of analytical results to the Permits Project Manager, latitudes and longitudes (using NAD 83) for station locations must be provided to the Corps by the applicant.

## 4.2 Sample Collection

1. Sediment samples must be collected according to the approved SAP. In the instances where vertical grain size homogeneity exists and the project depth is less than 2 feet, a grab sampler can be used **if agreed by the Corps prior to field sampling**. A core sampler should be employed in all other cases to ensure the samples are representative of materials to dredging depth, including expected overdepth. To ensure an adequate sample is representative of a project, NAE must approve the sampling apparatus. The type of equipment used to collect the samples should be noted as part of the project record. For example, if coring was used, the type of corer (gravity, vibracore, split spoon, borings, etc.) and the core liner (polycarbonate or butyrate, etc.) should be added to the field documentation. Core logs should be provided, along with narratives describing relative grain sizes, color, odor, strata, core length and depth of penetration along with other pertinent sediment sampling observations.

2. In instances where significant (at least 2.0 ft) vertical stratification is evident in samples, subsampling and testing of each layer may be required to adequately characterize the materials. If the stratum is less than 2 ft, then the sample may not be large enough to meet minimum sample requirements for physical and chemical analyses. A wider diameter core or a grab, for example, will provide for a larger sample. Information may be necessary for disposal management decisions, such as the necessity for capping and the availability and volume of uncontaminated capping materials from within the project site. Decisions as to the need and type of vertical subsampling must be coordinated with NAE. If there is no means or time for the field crew to communicate with NAE, the subsamples should be taken back to the lab and analyzed for grain size, so that NAE and the applicant can determine if the cores should be vertically composited. In this case, collection of extra sediment within each stratum is advised to ensure an adequate sample to run additional analyses if segmentation is warranted. **The goal is to provide the best possible characterization of the material to avoid overestimating the amount of contaminated material that may require special and likely more expensive disposal considerations.**

3. In situations where grain size analyses show samples to be comparable and samples represent a similar project segment(s), compositing of samples could occur. In all instances where compositing is contemplated and NAE has required grain size analyses, NAE must review grain size data prior to any compositing, **and will make the final decision on any compositing scheme.** Should compositing be allowed, the individual samples making up the composites must be archived by the testing laboratory until results of analyses have been reviewed by NAE.

4. Care should be taken to avoid contamination from sampling gear, grease, ship winches or cables, airborne dust, vessel engine exhaust, cross contamination and improper subsampling procedures. Engines should be shut off during sampling, if possible. If not possible (due to boat traffic, type of workboat, currents, etc), then the sampling effort should be performed upwind of the exhaust. It is recommended that core extrusion and sample mixing be performed in the laboratory. However, if on-board mixing is necessary, this effort must be performed away from exhaust fumes and any other sources of contamination. In addition, care must be taken to avoid cross contamination. All core samplers or other sampling devices must be appropriately decontaminated between samples.

The applicant must ensure that the workboat has room to store cores vertically out of the way from contamination and disturbance.

5. A sufficient sediment mass must be collected to meet the objective(s) of the sampling program. A minimum of approximately 1000 grams of sediment per sample must be collected for bulk physical and chemical analyses. The mass of sediment will vary with grain size, density, core depth/diameter and should be assessed before sampling to ensure adequate mass. It should be noted other types of analyses require higher masses, e.g., bioaccumulation test which needs a minimum of 7,500 grams (see Chapter 7, Section 7.2). Material must be available for analyses and for partitioning of samples to meet archiving requirements cited in Table 5 of the EPA/Corps document entitled *QA/QC Guidance for Sampling and Analysis of Sediments, Water and Tissue for Dredged Material Evaluations, Chemical Evaluations* (EPA/USACE, 1995). The guidance specified in *Methods for Collection, Storage, and Manipulation of Sediments for Chemical and Toxicological Analyses* (EPA 2001d) should also be consulted.

6. The project sampling must be taken at the precise locations required by the NAE approved SAP. Vessel positioning must be determined, using any number of techniques including GPS, Loran and surveying equipment. GPS systems need to be calibrated using known references. In all cases, NAE requires that **sample location** latitudes and longitudes be **recorded and provided to NAE** for each sampling location for compatibility with the NAE regional database. All data should be reported in NAD 83 decimal minutes. Locational information for each sampling point should be recorded in-field on a Station Location Log, Sediment Sampling Log or similar document and be included as part of the data QA/QC portion of the analytical results. Examples of these types of documents are included in Appendix A of EPA/USACE (1995).

## 4.3 Sample Handling, Preservation and Storage

1. The applicant is responsible for ensuring that the sampling, handling and preservation and storage procedures and the applicable quality assurance/quality control measures are followed for both sampling and analysis. These procedures must be adequately described in the approved SAP and the LQAP. The LQAP must be on file with the Corps in order for test data and analysis to be

accepted. These Plans are based on the EPA/USACE QA/QC Guidance Manual dated 1995. The guidance specified in *Methods for Collection, Storage, and Manipulation of Sediments for Chemical and Toxicological Analyses* (EPA 2001d) should also be consulted.

2. Samples are subject to chemical, biological and physical changes as soon as they are collected. It is therefore imperative that, from initiation of collection activities until samples are analyzed, all applicable Quality Assurance and Quality Control (QA/QC) procedures are followed.

3. Sample preservation should be accomplished onboard the collecting vessel whenever possible. If final preservation cannot occur onboard, an interim preservation technique that preserves the sample integrity should be employed. Onboard refrigeration can be accomplished with coolers and ice while samples that are to be frozen can be placed in coolers with dry ice. Sediment samples for biological analysis should not be frozen but preserved at 4° C. No samples should be allowed to dry. Additional information is given in Chapter 8 of the Green Book (EPA/USACE 1991) or the QA/QC guidance manual (EPA/USACE, 1995).

4. In general, careful choice of sampling gear and containers should be made for each group of chemicals analyzed to avoid sample contamination. Prior to contact with samples, equipment and containers should be cleaned and rinsed. Specific methodologies and containers are discussed in EPA (2001) and EPA/USACE (1995). Labels for the containers must be able to withstand environmental extremes and remain legible.

5. Sample containers should be filled to the top unless the sample is to be frozen, in which case room for expansion must be allowed. If subsamples are to be taken from the container, the container is best left about 3/4 full to allow for proper stirring.

6. Work should start as soon as possible on sediments so as not to exceed the holding time requirements (as exhibited in EPA/USACE (1998)). The time between sample collection and analysis should be minimized to maintain the integrity of the sample. The longer the sample is stored, the more difficult it becomes to accurately assess sample results. For example, over time a relatively low contaminated sample can become increasingly toxic to bioassay organisms (due to ammonia or other constituents).

## 4.4 Sampling of Reference Sediments, Control Sediments and Water

Test procedures are conducted on the control and reference sediments in the same way as on the dredged material proposed for open water disposal.

A. REFERENCE AND CONTROL SEDIMENTS

1. Sample handling, preservation and storage QA/QC requirements (see EPA/USACE 1998) are the same for reference and control sediments as those for the dredged material. Reference samples may be collected with grab samplers.

2. Reference sampling sites are determined through a Region I EPA and NAE cooperative program that designates reference locations for each active disposal site. Current reference sampling sites will be indicated in the approved SAP. The location of reference sampling sites for each established disposal site are shown below. Testing laboratories are responsible for collection of control sediments.

3.

| | | |
|---|---|---|
| Rockland | 44° 7.1' N | 68° 58.70' W |
| Portland | 43° 38.6' N | 69° 59.01' W |
| Cape Arundel | 43° 17.9' N | 70° 26.02' W |
| Massachusetts Bay | 42° 22.70' N | 70° 30.30' W |
| Cape Cod Bay | 41° 57.50' N | 70° 16.00' W |
| New London | 41° 16.7' N | 72° 02.0' W |
| Cornfield Shoals | 41° 15.63' N | 72° 13.32' W |
| Central Long Island Sound (LIS) | 41° 8.1' N | 72° 50.06' W |
| Western LIS | 41° 58.69' N | 73° 29.20' W |

B. WATER

1. Should water samples be necessary to prepare sediment elutriate samples, they should be collected with either a non-contaminating pump or a discrete water sampler. Samples for preparation of the elutriate should be collected within the proposed dredging site, at mid-water depth at a location(s) in the vicinity of the sediment samples, but avoiding any outfalls or other sources of pollution. For disposal sites < 30 ft depth, one sample mid-depth will be collected. For sites > 30 ft depth, the sample should be a composite of near surface, mid-depth and near bottom samples (3 ft above the bottom).

2. Control water is analogous to control sediment as it is used for water-column bioassay control treatments. Control water should be the same water in which the test organisms were held prior to testing. Field collection of the water (if collected during the same field sampling as sediment samples), should be done prior to sediment sampling and at near-bottom depths. The control water may be clean seawater or adequately aged artificial seawater.

3. Quality Assurance/Quality Control procedures need to be followed for sampling and analysis.

**4.5 Sample Documentation**

1. A complete field record of all procedures must be maintained including station locations, sample handling, preservation and storage procedures. Any circumstances potentially affecting the sampling must be noted as they may prove invaluable in explaining a data anomaly.

2. The following information represents the minimum that must be placed on a sample label.

   - Unique identifying code
   - Location (station number) and depth
   - Analysis or test to be performed
   - Preservative and/or storage method
   - Date and time of collection
   - Special remarks if appropriate

- Initials or name of person doing the collecting

3. The information on the sample label represents the first step in a sample tracking (chain of custody) procedure. This procedure for tracking samples from collection through completion of analyses has to be in place prior to the initiation of sampling, with appropriate personnel assigned responsibility for the tracking and sample custody. Example sample labels and Chain of Custody forms are provided in Appendix A of EPA/USACE (1995).

4. Results of analyses submitted to NAE for evaluation must be made using the NAE formatted data template available on the Corps NAE website or as a computer disk. Additionally, the applicant must provide completed QC Summary Tables (Appendix II, also available on this website or disk) and hard copy results of the QC analyses. This format is necessary to facilitate the project review process and to ensure completeness of the submittal. Project data not submitted in this format will be considered incomplete and a resubmittal will be required. The format is provided to the applicant by the Corps when the SAP is approved.

5. As part of the chain-of-custody procedure and insuring an accurate evaluation of test results, sample designations used to identify sample locations in the field must be maintained throughout the process from sampling to data presentation. Records should include field log books, location of samples (latitude, longitude), positioning technology, sample labels, records of containers, time and conditions of storage. All sample containers and storage conditions must comply with the specifications in the Green Book, ITM, the "QA/QC Guidance for Sampling and Analysis of Sediments, Water and Tissues for Dredged Material Evaluations, Chemical Evaluations" (EPA/USACE 1995) and EPA (2001). Records must be kept a minimum of 5 years.

# 5.0 PHYSICAL AND CHEMICAL ANALYSIS OF SEDIMENTS

Testing is commonly required to characterize the physical and chemical properties of sediments proposed for dredging. The following information supplements Section 9.0 of the National Ocean Disposal (EPA/Corps 1991) ("the Green Book") and Inland Testing (ITM) (EPA/USACE 1998) guidance manuals and the QA/QC guidance manual (EPA/Corps 1995).

## 5.1. Initial Characterization of Sediment

All individual core samples must be visually inspected prior to their extrusion from the core liner in preparation for subsampling, homogenization or compositing. Each core must be described in terms of any discernible sediment strata characterized by changes in composition, texture, grain size, color and odor (e.g., sulfides, oil).

If **no significant strata** (< 2 ft thick) are present (as visible in a clear core liner), sediment in the core liner should be extruded, thoroughly homogenized and samples taken for physical parameters listed in Tables 1 and 4 (if necessary). If split spoons or other coring devices are used, the core must be extruded to examine and describe it. A subsample representative of each core must be archived in case additional or repeat analyses are required. As discussed in Chapter 4, should **any significant strata be present**, the applicant must contact the New England District (NAE) immediately for a determination of the need for analyses of individual strata prior to any further manipulation of the core. Archiving the sample in this instance is also required.

Sediment proposed for dredging and reference sediments must be analyzed for grain size distribution, total organic carbon (TOC) and total solids/percent moisture (Table 1). In addition, specific gravity, bulk density and Atterberg limits may be required on a case-by-case basis and are described in Section 5.3.

The grain-size analysis must be conducted according to the methods described in Plumb (1981) or ASTM (1998 a) and reported as percentages retained by weight in the following size classes at a minimum:

Gravel
Coarse Sand
Medium Sand
Fine Sand

Silt/Clay (expressed as "Fines")

Gravel and sand fractions should be separated using the standard sieve sizes in Table 1 (ASTM 1998 a, D 422-63). In addition to reporting the percentages of each size class, the applicant must graph the cumulative frequency percentages using the Engineering (ENG) Form 2087 or a similar form (Figure 4). There may be cases where silt and clay fractions will need to be distinguished. The NAE will provide guidance on whether it is needed on a case-by-case basis. These will be determined on a case-by-case basis. Both silt and clay fractions should be quantified by hydrometer (ASTM 1998 a), pipette or Coulter Counter (Plumb 1981). Further analysis of other size classes may be required to evaluate suitability for beneficial use or other purposes.

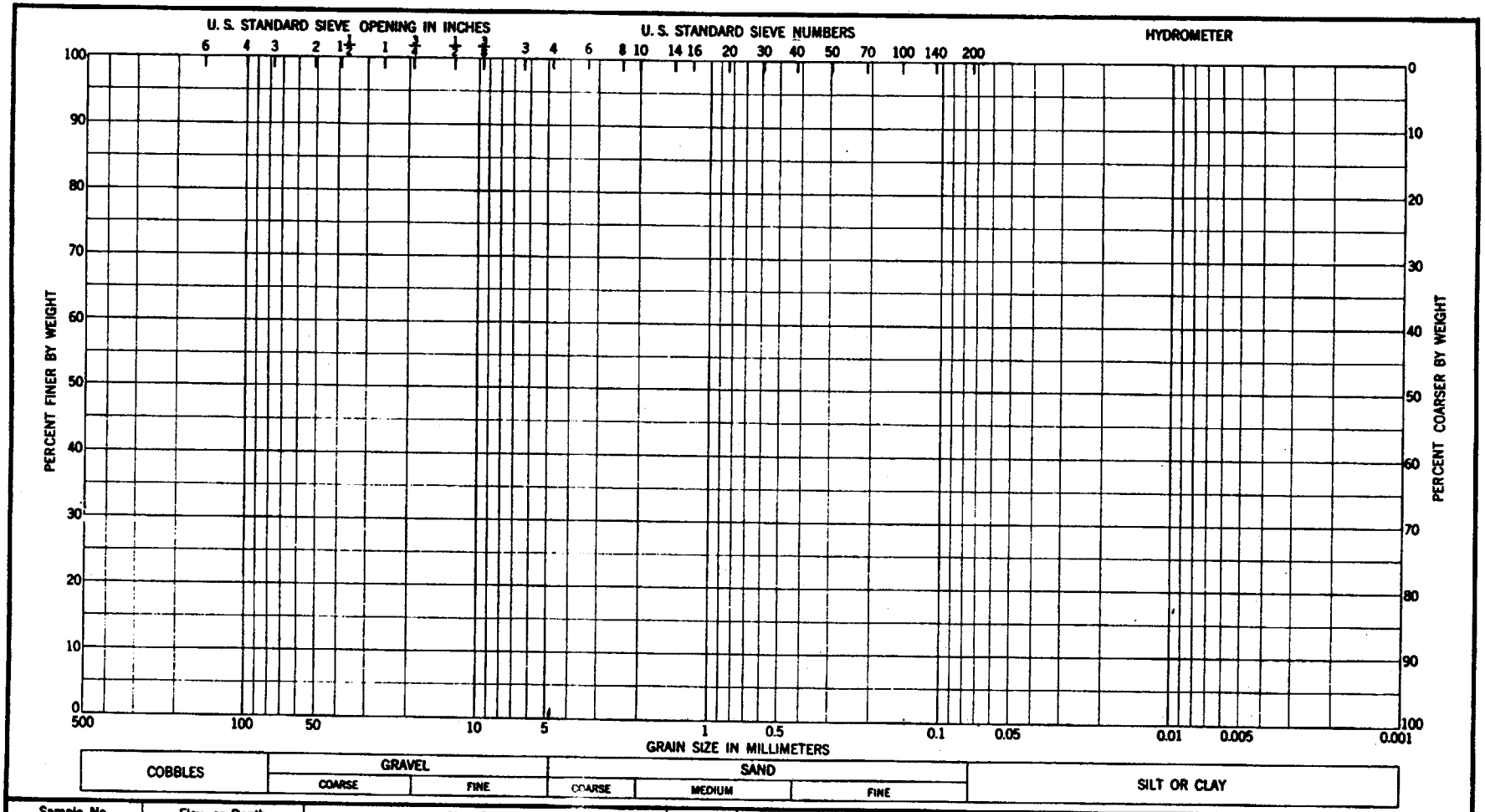
Measure the **total solids** and calculate **percent moisture** as described by Plumb (1981) or APHA (1995).

Note that the results of the above physical analyses *may* be used to support compliance with one or more of the three exclusionary criteria in 40 CFR 227.13(b) for ocean disposal or support a determination that the material is not a carrier of contaminants under 40 CFR 230.60 (a) for other aquatic disposal. If physical analyses show that the dredged material meets one or more of the exclusionary criteria, *and* if other pertinent, historical, and site-specific information can support the criteria, the material may be approved for disposal without further testing.

**5.2. Chemical Analysis of Sediment**

The chemicals of concern routinely required are listed in Tables 2 and 3. Table I-1 in Appendix I lists additional project-specific contaminants of concern. The routine metals, PCBs, PAHs and pesticides listed in Tables 2 and 3 were chosen based on their toxicity, their persistence in the environment, their ability to bioaccumulate and their widespread and consistent occurrence in New England estuarine, marine and freshwater sediments and organisms.

The target quantitation limits (TQLs) listed in the Tables are performance goals set between the lowest, technically feasible quantitation level for routine analytical methods and available background concentrations at reference areas in the vicinity of the disposal sites. As a routine data acceptance criteria, the Method Detection Limits (MDL, see below) for each analyte must be below the listed TQLs, with the caveat that some sediments with higher % moisture content may have MDLs higher than the TQLs. MDLs are calculated using the method described in Section 5.4 (c) and must be




| Sample No. | Elev or Depth | Classification | Nat w % | LL | PL | PI |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

GRADATION CURVES

Project

Area

Boring No.

Date

ENG FORM 1 MAY 63 2087

GPO 926-250

**FIGURE 4. SEDIMENT GRAIN SIZE GRADATION GRAPH**

performed at a minimum every six months (see below). Achieving the TQLs is critical to providing a consistent and accurate quantitation of contaminants of concern and a valid comparison with known background concentrations in New England estuarine and marine sediments in reference areas for disposal sites.

As noted in Tables 2 and 3, the specified methods (listed from the Green Book, ITM, QA/QC manual (EPA/USACE 1995) and the EPA guidelines on clean metals techniques (EPA 1996a,b,c,d)) are not required, as other acceptable methods are available. Whichever method is used, it is the applicant's responsibility to meet the target quantitation levels and the specified performance standards in Tables II-1 through II-5, the attached QC Summary Tables (Appendix II). These performance standards assess accuracy, as measured by standard reference material, and precision, as measured by duplicates and matrix spike duplicates, for the contaminant groups listed in Tables 2, 3 and Appendix I. Each applicant must demonstrate that any new lab they choose can meet these specifications prior to the analysis of any samples by the approval of an LQAP (see Section 4). Some labs have had difficulties in the past meeting the required target quantitation levels because of use of inappropriate sample preparation and clean-up procedures to remove interfering substances typically found in marine sediments (e.g., sulfides). Appropriate sample preparation, clean-up and analytical methods have been developed for estuarine/marine sediments by NOAA (1993) and EPA research laboratory at Narragansett, RI (EPA 1993) that have successfully met the TQLs. These are available from EPA Region I upon request. If the TQLs required quantitation limits cannot be attained, a detailed explanation must accompany the data providing the reasons for not attaining the required quantitation limits. Re-analysis may be necessary.

The concentration and analytical detection limit for each of the following analytes on a dry weight basis should be reported as: ppm for metals, ppb for organics, parts per trillion (pptr) for dioxins/furans and dioxin-like PCBs. Percent solids, used to calculate dry weight concentrations, must also be reported. The format for reporting is discussed in Section 4.5.

As discussed in the Green Book (Section 9.3.2), capillary gas chromatography with electron capture detection is recommended for analysis of PCBs and pesticides, whereas GC/MS in the Selected Ion Monitoring (SIM) mode is recommended for the PAHs and other semi-volatiles to meet the TDLs. Second column confirmation of pesticides is required. Such confirmation for PCBs is recommended but not required at this time. The 18 PCB congeners (listed in Table 3) are those analyzed in the NOAA

National Status and Trends Program (NOAA 1991). Additional congeners such as the non-ortho, mono-ortho and di-ortho dioxin-like PCBs (e.g., PCB # 77, 126, 169) may be required when dioxin is a contaminant of concern.

**Total organic carbon (TOC)** (Table 3) must be determined on all samples and subsamples. TOC must be analyzed in duplicate in addition to an SRM or laboratory control sample (LCS).

The Corps may require analysis of additional contaminants of concern other than those listed in Tables 2 and 3 as they are identified in the Tier I review. These remaining pollutants and other potential contaminants of concern and acceptable TQLs are listed in Appendix I. Required analyses will be documented in the approved SAP.

As a general rule, Gas Chromatography/Mass Spectroscopy (GC/MS) chromatograms must be scrutinized for the presence of compounds not included on the target analyte list. These compounds must be tentatively identified and always reported.

**5.3. Additional Physical Characterization of Sediment**

Additional characterization of the sediments may be required on a case-by-case basis, for modeling and geotechnical evaluations. These include specific gravity, bulk density and Atterberg Limits (Table 4). **Specific gravity** should be measured following APHA (1995), ASTM (1998 b, D 854-92) or Plumb (1981). **Bulk density** of sediment should be determined according to Klute (1986) or DOA (1980). **Atterberg Limits** may be required to assess the relative cohesiveness of the sediment. The procedures are outlined in ASTM (1998 c, D 4318-95)I. The **plastic/liquid limits** and **plasticity index** must be reported on ENG Forms 3838 and 4334 (Appendix II), respectively, or a facsimile.

**5.4. Quality Control Measures**:

The following analytical quality control measures must be followed for the above referenced methods. They are explained in more detail in Quality Control (QC) Summary sheets (Tables II-1 through II-7, Appendix II). Along with reporting the data generated from the sediment analyses, the applicant's contractor laboratory is required to document specified quality control measures in these attached worksheets. All QA/QC for Dioxin/Furan analyses (listed in Appendix I-1) will be documented according to the methods described in EPA Method 1613.

(a) Physical Analyses: The following QC checks are required for physical analyses (grain size, total solids) of sediments:

- Sample duplicate

(b) Chemical Analyses: The following QC checks are required for chemical analyses of sediments:

- Initial calibration
- Calculation of MDLs
- Blind analysis of spiked or performance evaluation material for calibration verification
- Continuing calibration checks
- Analysis of Reference Materials or Laboratory Control Materials
- Method blank
- Matrix Spike
- Matrix Spike Duplicate
  Analytical replicates
  Internal standards
  Surrogates

(c) Detection/Quantitation Limits: The detection/quantitation limits used in this manual are defined as follows:

Method Detection Limit (MDL) is defined as:

A statistical determination based on measured variance that defines the minimum concentration of a substance that can be detected with 99% confidence that the analyte concentration is greater than zero. In other words, that the analyte can be qualitatively detected above signal noise. Quantitative measurement at the MDL is inaccurate and therefore data reported less than the Reporting Limit (RL), see below) and greater than or equal to the MDL should be qualified with a "J" as estimated. **Any analytes not detected (below the MDL) should be reported as one half the MDL and qualified with a "U".** Detection limits are analyte- and matrix-specific and may also be instrument- and laboratory-dependent (see below).

The procedure described below, based on 40 CFR Part 136, Appendix B, must be followed to verify the MDL for samples collected for each approved Sampling and Analysis Plan. This MDL verification must be submitted with the data or performed on a similar matrix within the previous six months.

Select one representative relatively uncontaminated sample for each matrix and spike it with the analytes of concern so that the resulting concentration is

between 1 and 5 times the TQLs listed in Tables 2, 3, 5, 8 and Table I-1 (Appendix I). Prepare and analyze a total of seven spiked replicates of the chosen representative sample. Calculate the sample standard deviation (in concentration units) of the seven measurements for each analyte of concern. This value must then be multiplied by 3.143 and reported as the MDL.

Target Quantitation limit (TQL):

The TQL is a performance goal set between the lowest, technically feasible detection limit (i.e., MDL) for routine analytical methods and available background concentrations at reference areas in the vicinity of the disposal sites. The goal is to have confidence in measured values in sediment, tissue or water at concentrations typical of areas near but unaffected by the disposal site or other pollution sources.

Practical Quantitation Limit (PQL):

The minimum concentration of an analyte or category of analytes in a specific matrix that can be identified and quantified above the MDL and within specified limits of precision and bias during routine analytical operating conditions.

Reporting Limit (RL):

The Reporting Limit (RL) is the sample-specific PQL adjusted for sample processing volumes and factors (such as dilution) which can raise or lower the PQL. The RL should not be higher than the TQL and is usually 3-5 times higher than the MDL. However, when sediments of higher % moisture are analyzed, the RL may be higher than the TQL.

(d) The applicant must submit documentation of all quality control measures performed during analysis of the samples. If any of the control limit criteria are exceeded, the sampling results may not be accepted.

**5.5 Data Reporting**

All applicants are required to submit physical and chemical bulk sediment data in the New England District (NAE) format that NAE will use to review its analyses. These formats are available on the NAE webpage (xxx ) or available on a floppy disk from the Corps contact listed in Section 1. These data must be supplied both as a hard copy and on a 3 1/2" floppy disk. The format will be provided by the Corps with the approved SAP. Non-detects should be reported at one half the MDL. The applicants may submit their own data summaries and analyses; however, they must also submit the original data and copies of sampling logs so that the Corps and EPA can conduct independent

analyses. All submitted data must be clearly presented and traceable to the original samples and subsamples. No permit will be issued based solely on an applicant's data analysis.

**TABLE 1. Parameters used for the physical characterization of sediments.**

| Parameter | Method | Measure/Quantitation limit |
|---|---|---|
| Grain Size Distribution | Plumb 1981; ASTM 1998 a | |
| Gravel (> 4.75mm) | | Retained on No. 4 Sieve |
| Coarse Sand (2.0-4.75mm) | | Passing through No. 4 and retained on No. 10 Sieve |
| Medium Sand (0.425-2.0mm) | | Passing through No. 10 and retained on No. 40 Sieve |
| Fine Sand (0.075-0.425mm) | | Passing through No. 40 and retained on No. 200 Sieve |
| Silt (0.005-0.075mm) | | As determined by Hydrometer, Pipette or Coulter Counter. |
| Clay (< 0.005mm) | | As determined by Hydrometer, Pipette or Coulter Counter. |
| Total Solids/ Water Content | Plumb 1981 | 1.0% |
| Total Organic Carbon (TOC) | Plumb 1981, EPA 1992, Puget Sound Method (PSEP 1986) | 0.1 % |

**TABLE 2. Metal contaminants of concern, analytical methods and target quantitation limits (dry weight) routinely analyzed in sediments.**

| Metal | Analytical Method[1] | Target Quantitation Limit (ppm) |
|---|---|---|
| Arsenic | 6010B, 6020, 7060, 7061 | 0.5 |
| Cadmium | 6010B, 6020, 7130, 7131 | 0.1 |
| Chromium | 6010B, 6020, 7190, 7191 | 1.0 |
| Copper | 6010B, 6020, 7210 | 1.0 |
| Lead | 6010B, 6020, 7420, 7421 | 1.0 |
| Mercury | 7471 | 0.02 |
| Nickel | 6010B, 6020, 7520 | 1.0 |
| Zinc | 6010B, 6020, 7950 | 1.0 |

[1] The specified methods are recommendations only. Other acceptable methodologies capable of meeting the TQLs can be used. Sample preparation methodology (e.g. extraction and cleanup) and sample size may need to be modified to achieve the required target quantitation limits.

**TABLE 3. Organic contaminants of concern, analytical methods and target quantitation limits (dry weight) routinely analyzed in sediments.**

| Chemical Constituent | Analytical Method[1] | Target Quantitation Limit[2] |
|---|---|---|
| **TOTAL ORGANIC CARBON (TOC)** | Plumb 1981, EPA 1992, Puget Sound Method | 0.1 % |
| **PAHs** | 8270C-SIM | 20 ppb[2] |
| Acenaphthene | | |
| Acenaphthylene | | |
| Anthracene | | |
| Benzo(a)anthracene | | |
| Benzo(a)pyrene | | |
| Benzo(b)fluoranthene | | |
| Benzo(k)fluoranthene | | |
| Benzo(g,h,i)perylene | | |
| Chrysene | | |
| Dibenzo(a,h)anthracene | | |
| Fluoranthene | | |
| Fluorene | | |
| Indeno(1,2,3-c,d)pyrene | | |
| Naphthalene | | |
| Phenanthrene | | |
| Pyrene | | |

**TABLE 3. Organic contaminants of concern, analytical methods and target quantitation limits (dry weight) routinely analyzed in sediments (continued).**

| Chemical Constituent | | Analytical Method[1] | Target Quantitation Limit |
|---|---|---|---|
| **PESTICIDES** | | NOAA (1993), 8081B | 1 ppb[2] |
| Aldrin | | | |
| cis- and trans-Chlordane | | | |
| cis- and trans-Nonachlor | | | |
| Oxychlordane | | | |
| p,p'-DDT, DDE, DDD | | | |
| Dieldrin | | | |
| Endosulfan I and II | | | |
| Endrin | | | |
| Heptachlor | | | |
| Heptachlor epoxide | | | |
| Hexachlorobenzene | | | |
| Lindane | | | |
| Methoxychlor | | | |
| Toxaphene | | | 50 ppb |
| **PCB CONGENERS[3]** | | NOAA (1993), 8082A | 1 ppb[2] |
| 8* | 2,4' diCB | | |
| 18* | 2,2',5 triCB | | |
| 28* | 2,4,4' triCB | | |
| 44* | 2,2',3,5' tetraCB | | |
| 49 | 2,2',4',5 tetraCB | | |
| 52* | 2,2',5,5' tetraCB | | |
| 66* | 2,3',4,4' tetraCB | | |
| 87 | 2,2',3,4,5' pentaCB | | |
| 101* | 2,2',4,5,5' pentaCB | | |
| 105* | 2,3,3',4,4' pentaCB | | |
| 118* | 2,3',4,4',5 pentaCB | | |
| 128* | 2',3,3',4,4' pentaCB | | |
| 138* | 2,2',3,4,4',5' hexaCB | | |
| 153* | 2,2',4,4',5,5' hexaCB | | |
| 170* | 2,2',3,3',4,4',5 heptaCB | | |

| | |
|---|---|
| 180* | 2,2',3,4,4',5,5' heptaCB |
| 183 | 2,2',3,4,4',5'6 heptaCB |

**TABLE 3. Organic contaminants of concern, analytical methods and target quantitation limits (dry weight) routinely analyzed in sediments (continued).**

| Chemical Constituent | | Analytical Method[1] | Target Quantitation Limit |
|---|---|---|---|
| PCB CONGENERS[3] (continued) | | NOAA (1993), 8082A | 1 ppb[2] |
| 184 | 2,2',3,4,4',6,6' heptaCB | | |
| 187* | 2,2',3,4',5,5',6 heptaCB | | |
| 195* | 2,2',3,3',4,4',5,6 octaCB | | |
| 206* | 2,2',3,3',4,4',5,5',6 nonaCB | | |
| 209* | 2,2',3,3',4,4',5,5',6,6' decaCB | | |

[1] The specified methods are recommendations only. Other acceptable methodologies capable of meeting the TDLs can be used. Sample preparation methodology (i.e., extraction and cleanup) (EPA 1993; NOAA 1993) and sample size may need to be modified to achieve the required target quantitation limits.

[2] Applies to each analyte listed below unless otherwise noted.

[3] Total PCBs are to be estimated based on the following: Total = 2 X [sum of 18 NOAA summation congeners marked with *] (T.Wade, personal communication).

**TABLE 4. Additional parameters used for the physical characterization of sediments.**

| Parameter | Analytical Method | Measure/ Quantitation Limit |
|---|---|---|
| Specific Gravity | Plumb 1981<br>ASTM 1998 b<br>APHA 1995 | 0.01 |
| Bulk Density | Klute 1986<br>DOA 1980 | 0.01 $g/cm^3$ |
| Atterberg Limits | ASTM 1998 c | |
| Liquid Limit | | |
| Plastic Limit | | |
| Plasticity Index | | |

# 6.0 WATER COLUMN EVALUATION

## 6.1. Tier II. Compliance with Water Quality Criteria/Standards

The discharge of dredged material into the water column and resuspension at an aquatic disposal site may introduce sediment contaminants into the water column. As required in 40 CFR 227.6 (c)(1) and 40 CFR 230.10 (b) (1), the discharge must be in compliance with marine water quality criteria after allowance for mixing for discharges in federal waters and state water quality standards for discharges in state waters, if applicable. Based on 40 CFR 227.6, compliance with marine aquatic life water quality criteria or state water quality standards **must** be evaluated for every discharge in federal or state waters. The federal criteria are shown in Table 5. State water quality requirements for dredged material discharges vary with each state. Each appropriate state department of environmental protection office, in coordination with NAE, will assess compliance with applicable state standards using the data described below. General procedures for these analyses are described in Section 10.1 of the Green Book unless otherwise noted below.

Step 1: Criteria screen for compliance with EPA Water Quality Criteria

As a first step in evaluating compliance, the applicant may use the dry weight sediment concentrations of listed contaminants which assumes a total release from the sediments to the water column as described in Section 10.1.1 of the Green Book and Section 5.1 of the ITM. The model to be used is described below (6.4). As discussed in those sections, the analysis need only be run for the contaminant of concern that requires the greatest dilution for compliance. If the modeled discharge meets the criteria (Table 5), then no further analysis of water quality criteria (WQC) are needed. If the analysis shows that the discharge exceeds the criteria, then the standard elutriate test, as described in Step 2, must be performed.

Step 2: Standard Elutriate Analysis

The dredged-material elutriate preparation is conducted according to the methods presented in ITM Section 10.1.2.1: ("Standard Elutriate Test") with the following modifications (*italicized*). Samples for the elutriate and the water column toxicity test (Section 6.2) can be prepared from the same sediment-water mixture. To evaluate water quality criteria in the liquid phase, the elutriate water must be centrifuged to

remove particulates in accordance with the guidelines in Section 10.1.2.1 of the ITM. (The sample is not centrifuged in the case of the water column toxicity test since it assesses toxicity in the liquid and suspended solid phases (See ITM Section 11.1.4.)) The chemical analysis of the elutriate and dredging site water is discussed in ITM Section 9.4.2. Chemical Analysis of Water. *If "clean" seawater is used to prepare the elutriate and water column toxicity tests, then, for baseline purposes, the "clean" seawater must be analyzed for all the chemical parameters measured in elutriate and dredging site waters.*

Table 5 provides the recommended methods and required Target Quantitation Limits (TQLs) for each contaminant of concern. The reference methods in Table 5 should be consulted when selecting methods for water analysis. So-called "clean" techniques for sampling (EPA 1995a) and analyses of metals are currently available from EPA and are listed in Table 5. For extraction and analysis of PCB congeners, the NYDEC method (NYDEC, 1991) is also recommended. The 18 PCB congeners are listed in Table 3. If there is doubt about meeting TQLs, the applicant should contact New England District before any analyses are performed.

Particular note should be taken of the volume of the water samples required to meet the TQLs for water analysis (Table 5). As a general rule, at least 1 liter water samples are necessary for each organic analysis and 1 liter for metal analyses to provide TQLs that are below the applicable marine WQC. Larger samples are recommended since there should be enough left over in case repeat analysis is required. Additional clean-up steps also may be necessary, especially for the organics. It is important for a valid mixing evaluation (see Section 6.4 below) that accurate ambient contaminant concentrations be measured in the field collected ambient disposal site water samples. To meet the TQLs in Table 5 for the organics in the ambient samples, a larger sample may be necessary. An example procedure for collecting large field samples can be found in Appendix III.

At a minimum, chemical analysis must be conducted for the inorganic and organic analytes given in Table 5. Additional contaminants of concern may be requested for specific projects. Both elutriate (made up of dredging site water and sediments to be dredged) and disposal site water are to be tested in triplicate. Disposal site water values are used in the calculation to determine WQC compliance, or, existing data (provided by the Corps) in the vicinity of the disposal site may be substituted.

Refer to Green Book and the ITM (Sections 9.4): Chemical Analysis of Water and Section 10.0 of Guidance for Performing Tier II Evaluations and EPA/USACE (1995) for general guidance.

## 6.2. Tier III Water-Column Evaluations

Tier III water-column tests evaluate the potential for toxicity of the dissolved and suspended portions of the dredged material that remain in the water column after discharge of the dredged material. The Tier III water-column bioassays are run if the Tier II evaluations are inconclusive: i.e., there are no WQC for all contaminants of concern or there is reason to suspect additive or synergistic effects among the contaminants. The Tier III water-column tests involve exposing fish, pelagic crustaceans and planktonic invertebrate larvae to a dilution series containing dissolved and suspended components of the proposed dredged material. An overview of the Tier III water-column evaluations is presented in the Green Book and the ITM under Section 6.1 in both documents. Technical guidance for performing the tests is provided in the ITM Section 11.1: Tier III Water-Column Toxicity Tests. The NAE will specify to the applicant which species in Table 6 of this manual will be required for these tests.

Technical guidance on conducting water-column bioassays is provided in ITM Section 11.1: "Tier III: Water Column Toxicity Tests". Three series of tests are necessary; tests must be run using a fish (*Menidia* sp., *Cyprinodon variegatus*), a crustacean (*Americamysis bahia*) and a planktonic larvae (bivalve or echinoderm) (Table 6). The mysids should be fed as prescribed by EPA (1991b) or ASTM (1998 d,e). Bivalve larvae and silversides must not be fed (ASTM 1998 d,e,f). Test duration is generally 96 h except planktonic larvae which is 48 h. The procedure for preparing the water column toxicity test sample is given in Section 11.1.4 of the ITM with the following modifications (*italicized*). *In cases where the salinity of the dredging site water is detrimental to the health of the test organism (too low), all the toxicity water samples must be prepared using "clean seawater." The necessary dilutions may be made using water collected from clean seawater or aged artificial seawater.* Each series should include 100%, 50%, and 10% treatments and a 0% treatment (=100% dilution-water treatment). Clean seawater in which the organisms were held prior to testing must be run as a control. If the diluent is the same water the organisms are held in prior to testing, then the control and 0% treatment are one and the same. There is no reference site water in the water column toxicity test. Some fine-grained sediments can create turbidity in the test water even after settling. In this case, the ITM Section 11.1.4 allows mild centrifugation "...until the suspension is clear enough at the first observation time for the organisms to be visible in the testing chamber."

A **minimum** of five replicates per treatment concentration and a **minimum** of 10 organisms per replicate are required except for larvae (see next paragraph). As stated in the Green Book, the applicant should ensure that organisms are not overcrowded in the test chambers which can stress the organisms and falsely influence the results. The number of surviving fish and mysids for each replicate must be recorded at 0, 4, 24, 48, 72, and 96 h.

A minimum of five replicates per treatment are also required for the larvae bioassay. A suspension of fertilized eggs is used in the preparation of the test solutions. The suspensions containing bivalve larvae should contain 20-30 embryos/mL whereas the suspensions containing sea urchin larvae should contain 2000 embryos/mL. - Follow ASTM (1998 g) protocol for the bivalve water-column toxicity test or the procedure in EPA (1990) in Appendix V for sea urchin larvae. Use a light box or dissecting microscope to record the number of live animals. Use of an image analyzer as discussed in this procedure is not required here. For the larval test, centrifugation of a turbid supernatant is not necessary and should not be performed. The test is terminated in 48 hours. At this time, the larvae in the 0% treatment should have reached the appropriate stage of development (straight hinge--D shape for bivalves and plutei for the sea urchin).

For all test organisms, any sublethal effects such as physical or behavioral anomalies must also be reported. Daily water quality records must be kept for salinity, temperature, DO and pH for each test dilution.

**6.3. Quality Control Measures**

The EPA Region I and NAE require the following QC measures:

(a) Water Chemistry:

For water chemistry in the elutriate test, the analytical methods and TQLs described in Table 5 and EPA/USACE (1995), are recommended following the appropriate sample preparation. The analytical quality control measures described in each of the methods must be followed supplemented with applicable QA/QC guidelines described in Section 5.4 (b) and (c) for sediment chemistry. They are explained in more detail in Quality Control Summary Sheets Tables II-1-5, (Appendix II of this manual). Along with reporting the data generated from the chemical analyses, the applicant's contractor laboratory is required to document specified quality control measures in these attached worksheets.

(b) Water Column Toxicity tests: The EPA Region I and NAE require the following QC measures:

- All bioassays must be performed under the conditions specified in each of the test species sheets in Appendix V in either natural seawater or a synthetic seawater adjusted to salinity appropriate for the test species and disposal site (generally 25-30 $_{o/oo}$).

- The survival rate requirements in the Control treatments must be achieved. Failure to meet the applicable requirements below will likely invalidate the testing procedures and require retesting of the control and test samples.

- Control mortality requirements: ≤10% mean

  Control abnormality requirements:
  ≤ 30% for oyster and mussel larvae, or
  ≤ 40% for clam larvae)

(c) The applicant must submit documentation of all quality control measures performed during analysis of the samples. If any of the control limit criteria are exceeded, the data may not be accepted.

## 6.4. NUMERICAL MODELS FOR INITIAL-MIXING EVALUATIONS

This section explains describes how the Corps of Engineers uses numerical models to evaluate testing results from water column bioassays. The Corps and EPA will run the numerical models and make the evaluations; applicants or their agents do not need to run the models.

In general, initial-mixing evaluations for compliance with water quality criteria and toxicity will be performed by NAE as part of their assessment of each project. The following information supplements the national guidance in the ITM Appendix C: Evaluation of Mixing (EPA/ACE 1998) and Appendix B (EPA/USACE 1991).

Numerical models are components of the Tiers II and III water-column evaluations. The model used, STFATE, is contained in the Automated Dredging and Disposal Alternatives Management System (ADDAMS) from the ITM (updated software and is not referenced in the 1991 Green Book). However, this updated model is available for

unrestricted distribution from the U.S. Army Corps of Engineers Environmental Research and Development Laboratory (formerly the Waterways Experiment Station) web page (http://www.wes.army.mil/el/elmodels/index.html).

*The appropriate model is run only for the contaminant of concern that requires the greatest dilution.* If the contaminant requiring the greatest dilution is shown to meet the LPC, all of the other contaminants that require less dilution will also meet the LPC.

The STFATE initial-mixing model can be run on IBM®-compatible personal computers.

STFATE computes the movement of dredged material from an instantaneous dump and from a hopper dredge that falls as a hemispherical cloud. To properly apply this model, the total time required for the dredged material to leave the disposal vessel should not be greater than the time required for the material to reach the bottom. The model applies to both split-hull barge and hopper disposal.

This model accounts for the physical processes that determine the short-term fate of dredged material in the water column as it is disposed at open-water sites. The models assume that the dredged material behaves as dense liquid, and simulate the movement of the disposed material as it falls through the water column and spreads over the bottom. They do not account for resuspension or other long-term post-disposal phenomena on the water-column or benthic environment.

Input data for the models are grouped into the following general areas:

- Description of the disposal operation
- Description of the disposal site
- Description of the dredged materials
- Model coefficients
- Controls for input, execution, and output

ITM Appendix C: Evaluation of Mixing, Table C-2 lists each model's necessary input parameters and their corresponding units. Applicants must provide the following parameters: volume in barge, vessel course and speed, barge length and width, and post-disposal draft of barge. Additional descriptions and guidance for selection of values for many of the model parameters are provided in the Appendix C text and directly on-line in ADDAMS.

For discharge in federal waters, the results of the toxicity test will be used to determine compliance with the Limiting Permissible Concentration (LPC). The results of the water-

column tests are used to calculate the median lethal concentration ($LC_{50}$). The LPC for the dredged material is 1% of the $LC_{50}$. If the numerical mixing model predicts that the concentration of dredged material in the water column will not exceed 1% of the $LC_{50}$ concentration either outside the disposal site or within the disposal site 4 hours after discharge of the dredged material, the proposed discharge of dredged material meets the water-column LPC. If either of these criteria are not met, the dredged material does not meet the water-column LPC. For compliance of discharges in state waters, general guidelines are explained in Section 11.1.6 and Appendix C of the ITM. Here, the state environmental regulatory agency needs to be consulted to determine the mixing requirements for compliance with the water quality criteria in that state. Such mixing guidelines can vary with each state.

**TABLE 5. REQUIRED CONTAMINANTS, RECOMMENDED METHODS, TARGET QUANTITATION LIMITS AND FEDERAL WATER QUALITY CRITERIA USED IN WATER QUALITY CRITERIA COMPLIANCE DETERMINATION**

| CONTAMINANT | RECOMMENDED TEST METHOD[2] | TARGET QUANTITATION LIMIT(ug/L) | FEDERAL WATER QUALITY CRITERION (ug/L) |
|---|---|---|---|
| Metals[1] | | | |
| Arsenic | 200.9, 1632 | 1 | 69 |
| Cadmium | 200.9, 1637 | 1 | 42 |
| Chromium (VI) | 218.6, 1636 | 1 | 1100 |
| Copper | 200.9, 1639, 1640 | 0.6 | 4.8 |
| Lead | 200.9, 1639, 1640 | 1 | 210 |
| Mercury | 245.7, 1631, [3] | 0.4 | 1.8 |
| Nickel | 200.9, 1639, 1640 | 1 | 74 |
| Selenium | 200.9, 1639 | 1 | 290 |
| Silver | 200.9 | 0.5 | 1.9 |
| Zinc | 200.9, 1639 | 1 | 90 |
| Pesticides: | 3510B, 8081B[4] | | |
| Aldrin | | 0.26 | 1.3 |
| Chlordane | | 0.02 | 0.09 |
| Chloropyrifos | | 0.002 | 0.011 |
| Dieldrin | | 0.14 | 0.71 |
| 4,4' DDT | | 0.03 | 0.13 |
| a-Endosulfan | | 0.007 | 0.034 |
| b-Endosulfan | | 0.007 | 0.034 |
| Endrin | | 0.007 | 0.037 |
| Heptahlor | | 0.01 | 0.053 |
| Heptachlor Epoxide | | 0.01 | 0.053 |
| Lindane | | 0.26 | 1.3 |
| Toxaphene | | 0.04 | 0.21 |
| Industrial Chemicals: | | | |
| PCBs[5] | NYDEC, 3510B, 8082A | 0.006 | 0.03 |
| Pentachlorophenol | 3510B, 8270C | 2.60 | 13 |

**TABLE 5. REQUIRED CONTAMINANTS, RECOMMENDED METHODS, TARGET QUANTITATION LIMITS AND FEDERAL WATER QUALITY CRITERIA USED IN WATER QUALITY CRITERIA COMPLIANCE DETERMINATION** (continued)

[1] Determined as "total recoverable metals".

[2] Except for chromium and mercury, samples can be digested by Method 200.2 (EPA, 1991) and extracted by chelation/extraction as described under "Metals-14" S 9.2 (EPA, 1979, revised 1983), prior to analysis by Method 200.9. EPA Clean metal techniques (1600 series) are described in EPA (1995 a,b,c) and EPA (1996 a,b,c,d).

[3] Bloom and Crecelius (1983) method for determining mercury concentrations.

[4] Pesticides and PCBs can be extracted from the water by Methods 3510B and analyzed by Method 8081A (EPA 1986); PCB congener analysis by NYDEC (1991) are also recommended.

[5] Total PCBs will be estimated based on the summation of these congeners and using the equation: total PCBs = 2 X [sum of 18 congeners] (T. Wade, personal communication).

**TABLE 6. ORGANISMS REQUIRED FOR THE WATER COLUMN BIOASSAY[1]**

| Group | Organism | Scientific Name | Test Duration |
|---|---|---|---|
| I | Fish: | | 96h |
| | Silverside | *Menidia* sp. | |
| | Sheepshead minnow | *Cyprinodon variegatus* | |
| II | Mysid shrimp | *Americamysis bahia.* | 96h |
| III | Planktonic larvae: | | 48h |
| | Blue mussel | *Mytilus edulis* | |
| | American oyster | *Crassostrea virginica* | |
| | Hard clam | *Mercenaria mercenaria* | |
| | Coot clam | *Mulinia lateralis* | |
| | Sea urchin | *Arbacia punctulata* | |

[1] One type of organism must be tested from each group.

# 7.0 BENTHIC EFFECTS EVALUATION

The benthic effects evaluation involves whole sediment toxicity and bioaccumulation testing. The general procedures for Tier III toxicity tests are described in Sections 11.2 of the ITM (EPA/ACE 1998) and Green Book (EPA/USACE 1991) and freshwater testing manual (EPA 2000). Tier III bioaccumulation tests are described in Section 12.1 of the ITM and the freshwater manual (EPA 2000).

## 7.1 Tier III - Whole Sediment Toxicity Tests

The purpose of the sediment toxicity tests is to determine whether the sum of the sediment contaminants in combination with the physical characteristics will elicit a toxic response to exposed organisms after the material is deposited into the marine environment.

For marine and estuarine disposal, two test species of those listed in the Toxicity section of Table 7 are required –one of the three marine amphipod species (depending on salinity and grain size) and the mysid shrimp. Currently only one species is required for freshwater disposal. Species-specific test conditions are listed in Appendix V and are detailed in EPA (1994a) for estuarine/marine amphipods, EPA (1991b) for the mysid and Sections 11 and 12 of the freshwater testing manual (EPA 2000) for the freshwater amphipod and midge fly larva.

General guidance for the collection, handling and storage of sediments for biological testing are described in Section 4 of this manual and Section 8 of the Green Book. Section 8 of the EPA amphipod test manual (EPA 1994a) must be consulted for specific guidance related to the amphipod sediment toxicity tests. The Corps will specify any compositing of sediment samples that will be allowed in consultation with federal/state regulatory agencies.

Specific guidance on procedures for setting up, performing and breaking down the test is provided in EPA (1994a) for the amphipod species, and EPA (1991b) for the mysid species. All sediments tested must be press-sieved with a 1 or 2 mm sieve to remove unwanted debris and predators before being added to the test chambers. All data should be reported on the forms supplied in EPA (1994a, Appendix A, Figures A1-A5) or a close facsimile. In addition to the parameters on the forms, all observations on mortality, the formation of tubes or burrows, amphipod emergence from sediment, and any physical or behavioral abnormalities must be recorded.

Bulk sediment chemistry, for the project specific contaminants of concern, Total Organic Carbon (TOC) and grain size analyses may be required by NAE on subsamples of the sediments that are biologically tested. Subsamples of the dredged material, reference

and control sediments used in the test must be archived for possible future bulk analysis if the Corps or EPA requires them to be analyzed.

As a general rule, the applicant is required to seek approval from the Corps and EPA on project specific procedures for any sediments requiring treatment for ammonia toxicity. Amphipods and mysids are generally sensitive to sediment ammonia. Excessive ammonia concentrations may cause mortalities in these species. Because ammonia toxicity can generally change with ephemeral environmental conditions such as temperature, salinity, oxidation state and pH, excessive ammonia concentrations can confound the mortality endpoint of interest to the dredging regulatory program which focuses on more persistent toxics. To account for this potential false positive, the EPA and Corps have devised methods to reduce ammonia toxicity before any test begins (Sections 11.4.5-11.4.5.3 of the EPA amphipod manual (EPA 1994a), as amended by the "Errata" sheet for pages 80-82 of that document). Therefore, to avoid toxicity from ammonia, the applicant must insure that the sediment porewater total ammonia and un-ionized ammonia concentrations are below 20 mg/L and 0.4 mg/L, respectively before any amphipod is added to a test chamber. Collect porewater for ammonia and pH determinations at test initiation before the test organisms are added to exposure chambers. This will require setting up dummy chambers for porewater collection. Recommended procedures to collect porewater are described in Appendix VII. After treatment, the pore water concentrations must be maintained below the above values for 24 hours before the animals are added to the test chamber. Total and un-ionized ammonia levels must be monitored in the pore-water on days 1, 5 and 10 during the test. These measurements should be made in at least one chamber ("dummy" chambers for porewater collection) or using peepers (see Section 6.2.1 of EPA 2001d) for each homogenized sediment treatment level (control, reference, dredge site) tested. **All samples require triplicate analysis**.

For the mysid, *Americamysis (= Mysidopsis) bahia*, the applicant must follow the guidance in the June 14, 1994 memo to Mario Del Vicario from Elizabeth Southerland (Appendix VIII). Here, the concern is unionized ammonia in the overlying water (1 cm above the sediments). The applicant must insure that the water concentrations are below 0.6 mg/L at pH of 7.9-8.0 or 0.3 at pH of 7.5 before any animals are added to the test chambers. In this case overlying water is monitored each day.

As indicated in the Green Book and ITM, all control survivorship must be at least 90% for the test to be valid. The reader is referred to other QA/QC requirements in Section 7.3 of this manual.

**7.2 Tier III - Bioaccumulation Testing**

Bioaccumulation tests provide a measure of exposure of deposit-feeding marine animals to bioavailable sediment contaminants. In this case, representatives of a bivalve and a polychaete worm species are exposed for a 28 day period to dredging site, reference and control sediments. To clarify recommendations in the Green Book, the 28 day exposure test is required for organic contaminants of concern as well as for metals. General technical guidance is provided in Section 12.1 of the ITM (EPA/ACE 1998), Section 13 of the freshwater methods manual (EPA 2000) for freshwater disposal and Lee *et al.* (1989), as cited in the former documents.

The two required species for marine/estuarine disposal are listed in the Bioaccumulation section of Table 7– the sandworm, *Nereis virens*, and the bivalve *Macoma nasuta*. Each species must be exposed in separate aquaria because of the predatory behavior of *Nereis*. It should be noted that use of another set of aquaria will require a proportionally greater amount of sediments to be collected and processed. For freshwater disposal, the oligochaete, *Lumbriculus variegatus* is used.

All aquaria must have a sediment depth of at least 5 cm. At least 20 specimens of each species are required in each test chamber, although more may be necessary to conduct the prescribed tissue analyses at the end of the test exposure. It is the applicant's responsibility to insure that the laboratory provides enough animal tissue (size/number) to run subsequent chemical analyses. Generally, it is desirable to produce 50 g (wet weight) for each replicate and species. The number of animals and the size of the aquarium will vary with the size of individual animals acquired for the test. For the species in Table 7, tissue/sediment loading should not exceed 1 g tissue (wet weight minus shell) to 50 g sediment (wet weight) (Lee, EPA Newport Lab, personal communication). If dioxin/furan levels are required, then a separate set of aquaria may be required to provide adequate tissue for analyses to achieve the required TQLs.

Those constituents generally requiring analysis are listed in Table 8, but may include other contaminants as determined by the Tier I review and/or chemical testing of the sediments. The final decision on which project-specific contaminants are required is made by the Corps in consultation with other Federal/state regulatory agencies. Recommended tissue extraction and analytical methods are provided in NOAA (1993), EPA/USACE (1995) or EPA (1993). The applicant must insure the contracted laboratory can reasonably achieve the required TDLs listed in Table 8 and Appendix I, if applicable. The sample preparation methods for **animal tissue** described in EPA (1993) and EPA/USACE (1995) are highly recommended. As mentioned above, 50 grams of tissue (wet) per replicate is recommended (or enough to obtain acceptable TQLs). In addition to the contaminants, the lipids of each clam and worm tissue replicate should be analyzed using a modified Bligh and Dyer (1959) method developed by the U.S. EPA Narragansett Laboratory, (EPA 1995 d)(see Appendix IX). A copy of

this method is included as Appendix IX of this document. Percent water, solids and lipid must be reported for each species and replicate.

All appropriate QA/QC measures listed in Sections 9 and 12 of the ITM and EPA/USACE (1995) must be followed. Tissues of organisms randomly selected prior to initiation of bioaccumulation testing (pre-test analyses) must be analyzed and reported for all contaminants analyzed in the exposed organisms. A subsample of these pre-test samples of tissue of each species must be archived as the applicant may be required to analyze this tissue at a later date for specified contaminants.

As with toxicity tests, daily records must be kept of salinity, temperature, DO, pH, flow rate, obvious mortalities and any sublethal effects. Failure of organisms to burrow into the sediment or any other physical or behavioral abnormalities must also be recorded. All bivalves (whether pre or post-test) must be depurated for 24 hours in "clean seawater" prior to freezing. Polychaetes will not depurate in seawater alone and therefore require a 24 hour depuration with "clean sand."

**7.3 Quality Control Measures**

(a) The EPA Region I and NAE require the following biology QC measures:
All marine/estuarine bioassays must be performed under the conditions specified in each of the test species sheets in Appendix V in either natural seawater or a synthetic seawater adjusted to salinity appropriate for the test species and disposal site (generally 25-30 o/oo). Adherence with the applicable test acceptability requirements in EPA (1994a) must be documented for *Ampelisca abdita, Eohaustorius estuarius* and *Leptocheirus plumulosis* and in EPA (2000) for *Hyalella azteca.* Likewise, the QA procedures cited in the ITM and EPA (2000) must be followed and documented for bioaccumulation testing.

Bulk physical and chemical testing may be required for each sediment sample tested for biological analyses to insure the testing was performed on representative samples. This will be determined on a case by case basis.

The survival rate requirements in the Control treatments must be achieved. Failure to meet the applicable requirements below will likely invalidate the testing procedures and require retesting of the control, reference, and test samples.

Sediment toxicity control mortality requirements: $\leq$10% mean (amphipods control mortality $\leq$10% mean and no individual chamber $\geq$20% mortality)

Where control mortality >10% for sediment bioaccumulation samples, determine whether the following conditions exist: a) adequate replicates to obtain statistical power b) stressed organisms c) contaminated control sediment d) contamination of test system e) quality control problems f) adequate tissue for chemical analyses

(b) For tissue chemistry in the bioaccumulation testing, the applicant should follow similar QA/QC guidelines as described in Section 5.4 (b) and (c) for sediment chemistry. The analytical quality control measures in the above described methods must be followed supplemented with the guidelines described in Section 5.4 (b) and (c). The measures are explained in more detail in Quality Control Summary Sheet (Tables II-1 through II-8, (Appendix II). Along with reporting the data generated from the chemical analyses, the applicant's contractor laboratory is required to document specified quality control measures in these attached worksheets. All QA/QC for Dioxin/Furan analyses (listed in Appendix I-1) will be documented according to the methods described in EPA Method 1613.

(c) The applicant must submit documentation of all quality control measures performed during analysis of the samples. If any of the control limit criteria are exceeded, the data may not be accepted.

### 7.4 Statistical Analysis

Toxicity and bioaccumulation data should be analyzed as indicated in Appendix D of the "Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S --Testing Manual" (EPA/ACE 1998) (summarized in Table 9). As discussed in Appendix D, these methods are described in many popular general statistics texts such as Winer (1971), Steel and Torrie (1980), Sokal and Rohlf (1981), Dixon and Massey (1983), Zar (1984) and Snedecor and Cochrane (1989). In addition, Conover (1980) is recommended for nonparametric tests. Most of these tests are included in commercially available statistics software packages. Relative to detection levels, all undetected analytes must be reported in the data as one half the method detection level (MDL) as defined in section 5.4.

### 7.5 Data Reporting

All applicants are required to submit toxicity and bioaccumulation data in the New England District (NAE) format. The format will be provided by the Corps with the approved SAP. The appropriate QC Summary sheets described above must also be submitted with the data. The applicants may submit their own data summaries and analyses; however, they must also submit the original data and copies of sampling logs so that the Corps and EPA can conduct independent analyses. All submitted data must be clearly presented and traceable to the original samples and subsamples. No permit will be issued based solely on an applicant's data analysis.

**TABLE 7. ORGANISMS REQUIRED FOR THE WHOLE SEDIMENT TOXICITY AND BIOACCUMULATION TESTS[1]**

| Group/Taxa | | Habitat | Scientific Name | Test Duration |
|---|---|---|---|---|
| **TOXICITY** | | | | 10 d |
| I | Amphipod[1] | Marine/Estuarine and fine grain | *Ampelisca abdita* | |
| | | Estuarine | *Leptocheirus plumulosus* | |
| | | Marine/Estuarine and coarse grain | *Eohaustorius estuarius* | |
| | | Freshwater | *Hyalella azteca* | |
| II | Non-Amphipods | | | |
| | Mysid | Marine/Estuarine | *Americamysis bahia* | |
| | Midge larva | Freshwater | *Chironomus tentans* | |
| **BIOACCUMULATION** | | | | 28 d |
| I | Bivalve | Marine/Estuarine | *Macoma nasuta* | |
| II | Polychaete worm | Marine/Estuarine | *Nereis virens* | |
| | Oligochaete[2] | Freshwater | *Lumbriculus variegatus* | |

[1] One species from this grouping is required depending upon disposal site conditions
[2] Only one bioaccumulation test species is available and required for freshwater tests

**TABLE 8. TISSUE CHEMICAL CONSTITUENTS AND TARGET QUANTITATION LIMITS ROUTINELY USED FOR BIOACCUMULATION EVALUATIONS OF PROPOSED DREDGED MATERIAL.**

| Chemical Constituent | Analytical Method[1] | Target Quantitation Limit (wet weight) |
|---|---|---|
| TOTAL LIPIDS | EPA (1995c) | 0.1% |
| TOTAL WATER CONTENT | EPA (1986, 1987) | 0.1% |
| METALS | | ppm[2] |
| Arsenic | 200.8, 7061 | 0.5 |
| Cadmium | 200.8, 7131A | 0.1 |
| Chromium | 200.8, 7191 | 1.0 |
| Copper | 200.8, 7211 | 1.0 |
| Lead | 200.8, 7421 | 1.0 |
| Mercury | 7471 | 0.02 |
| Nickel | 200.8, 6010A | 1.0 |
| Zinc | 200.8, 7950 | 1.0 |
| ORGANICS | | |
| Pesticides | 8081B[2] | 1 ppb[2] |
| Aldrin | | |
| cis- and trans-Chlordane | | |
| cis- and trans-Nonachlor | | |
| Oxychlordane | | |
| p,p'-DDT, DDE, DDD | | |
| Dieldrin | | |
| Endosulfan I and II | | |
| Endrin | | |
| Heptachlor | | |
| Heptachlor epoxide Hexachlorobenzene | | |
| Lindane | | |
| Methoxychlor | | |
| Toxaphene | | 50 ppb |

**TABLE 8. CHEMICAL CONSTITUENTS AND QUANTITATION LIMITS ROUTINELY USED FOR BIOACCUMULATION EVALUATIONS OF PROPOSED DREDGED MATERIAL (continued).**

| Chemical Constituent | | Analytical Method[1] | Target Quantitation Limit (wet weight) |
|---|---|---|---|
| PCB Congeners[3] | | 8082A[2] | 0.5 |
| | ppb[2] | | |
| 8 | 2,4' diCB | | |
| 18 | 2,2',5 triCB | | |
| 28 | 2,4,4' triCB | | |
| 44 | 2,2',3,5' tetraCB | | |
| 52 | 2,2',5,5' tetraCB | | |
| 66 | 2,3',4,4' tetraCB | | |
| 101 | 2,2',4,5,5' pentaCB | | |
| 105 | 2,3,3',4,4' pentaCB | | |
| 118 | 2,3',4,4',5 pentaCB | | |
| 128 | 2',3,3',4,4' pentaCB | | |
| 138 | 2,2',3,4,4',5' hexaCB | | |
| 153 | 2,2',4,4',5,5' hexaCB | | |
| 170 | 2,2',3,3',4,4',5 heptaCB | | |
| 180 | 2,2',3,4,4',5,5' heptaCB | | |
| 187 | 2,2',3,4',5,5',6 heptaCB | | |
| 195 | 2,2',3,3',4,4',5,6 octaCB | | |
| 206 | 2,2',3,3',4,4',5,5',6 nonaCB | | |
| 209 | 2,2',3,3',4,4',5,5',6,6' decaCB | | |
| PAHs | | 1625C, 8270C, 8100, NOAA (1993)[2] | 20 ppb[2] |
| | Acenaphthene | | |
| | Acenaphthylene | | |
| | Anthracene | | |
| | Benzo(a)anthracene | | |
| | Benzo(a)pyrene | | |
| | Benzo(b)fluoranthene | | |
| | Benzo(k)fluoranthene | | |
| | Benzo(g,h,i)perylene | | |
| | Chrysene | | |
| | Dibenzo(a,h)anthracene | | |
| | Fluoranthene | | |

**TABLE 8. CHEMICAL CONSTITUENTS AND QUANTITATION LIMITS ROUTINELY USED FOR BIOACCUMULATION EVALUATIONS OF PROPOSED DREDGED MATERIAL (continued).**

| Chemical Constituent | Analytical Method[1] | Target Quantitation Limit (wet weight) |
|---|---|---|
| PAHs (continued) | 1625C, 8270C, 8100, NOAA (1993)[2] | 20 ppb[2] |
| Fluorene | | |
| Indeno(1,2,3-cd)pyrene | | |
| Naphthalene | | |
| Phenanthrene | | |
| Pyrene | | |

1 The specified methods are recommendations only. Other acceptable methodologies capable of meeting the TQLs may be used. Sample preparation methodology (e.g. extraction and cleanup) and sample size may need to be modified to achieve the required target quantitation limits.

2 Applies to each analyte listed below unless otherwise noted.

3 Total PCBs are to be estimated based on the following: Total = 2 X [sum of 18 NOAA summation congeners] (T. Wade, personal communication).

**TABLE 9. RECOMMENDED STATISTICAL METHODS FOR BIOLOGICAL TESTING[1]**

| Statistic | Method |
|---|---|
| Normality | Shapiro-Wilk's Test;<br>Kolmogorov-Smirnov (K-S) Test<br>Normality tests found in SYSTAT or SPSS |
| Equality of Variances | Bartlett's Test (should not be used to test equality of ranks)<br>Levene's Test<br>$F_{max}$ Test<br>Cochran's Test |
| Parametric | Fisher's Least Significant Difference (LSD) (if raw or transformed are normally distributed) in conjunction with analysis of variance (ANOVA). |
| Nonparametric | LSD on rankits (= van der Waerden's Test in Conover (1980)) (if the data converted to rankits are found to be normally distributed); or Conover T-Test (Conover 1980) (if the variances of the ranks are not significantly different); or<br><br>One tailed T-Test for unequal variances for each pair of treatments (if the ranks are significantly unequal). |

[1] Summarized from Appendix D (EPA/ACE 1998)

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# 9. APPENDICES

I. Additional Priority Pollutants of Concern and Target Quantitation Limits

II. Quality Control Summary Sheets

III. Forms for Atterberg Limits

IV. Procedures for Collection of Large Volume Water Samples

V. Sea Urchin Larval Toxicity Test Procedure

VI. Species-Specific Testing Condtions

VII. Pore Water Collection Procedure

VIII. Procedures for Addressing Ammonia Presence in *Mysidopsis* Sediment Toxicity Tests (Elizabeth Southerland Memo to Mario P. Del Vicario, dated June 14, 1994)

IX. AED Laboratory Operating Procedure, Measurement of Total Lipids using Modified Bligh-Dyer Method

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# APPENDIX I

## Additional Priority Pollutants of Concern and Target Quantitation Limits

# APPENDIX I.

TABLE I-1. Additional chemical constituents[1], EPA analytical methods and target quantitation limits used for the chemical examination of proposed dredged material and tissue for bioaccumulation testing.

| Chemical Constituent | Analytical Method | Sediment Target Quantitation Limit(dry wt) | Tissue Target Quantitation Limit (wet wt) |
|---|---|---|---|
| METALS | | (ppm) | (ppm) |
| Antimony | 7040, 7041 | 2.5 | 0.1 |
| Beryllium | 7090, 7091 | 2.5 | 0.1 |
| Selenium | 7740, 7741 | 1.0 | 0.2 |
| Silver | 7760 | 0.2 | 0.1 |
| Thallium | 7840 | 0.2 | 0.1 |
| MISCELANEOUS | | (ppm) | (ppm) |
| Cyanide | 9010, 9012 | 2.0 | 1.0 |
| Acid Volatile Sulfides | Allen et al. (1991) | 0.01 umol/g | N/A |
| Organotins | Uhler & Durrel (1989) Rice et al. (1987) | 10 ppb | 10 ppb |
| DIOXINS/DIBENZOFURANS | 8290, 1613 | (pptr) | |
| 2,3,7,8-TCDD/-TCDF | | 1 | 0.5 |
| 1,2,3,7,8-PeCDD/-PeCDF | | 5 | 0.5 |
| 2,3,4,7,8-PeCDF | | 5 | 5 |
| 1,2,3,4,7,8-HxCDD/-HxCDF | | 5 | 5 |
| 1,2,3,6,7,8-HxCDD/-HxCDF | | 5 | 5 |
| 1,2,3,7,8,9-HxCDD/-HxCDF | | 5 | 5 |
| 2,3,4,6,7,8-HxCDF | | 5 | 5 |
| 1,2,3,4,6,7,8-HpCDD/-HpCDF | | 5 | 5 |
| 1,2,3,4,7,8,9-HpCDF | | 5 | 5 |
| OCDD/OCDF | | 10 | 10 |

TABLE I-1. Additional chemical constituents[1], EPA analytical methods and quantitation limits used for the chemical examination of proposed dredged material (continued).

| Chemical Constituent | Analytical Method | Sediment Target Quantitation Limit(dry wt) | Tissue Target Quantitation Limit (wet wt) |
|---|---|---|---|
| | | (ppb) | (ppb) |
| WHO PCB CONGENERS | 1668[2] | 0.25[2] | 0.5[2] |
| PCB - 77 | | | |
| PCB - 81 | | | |
| PCB - 105 | | | |
| PCB - 114 | | | |
| PCB - 118 | | | |
| PCB - 123 | | | |
| PCB - 126 | | | |
| PCB - 156 | | | |
| PCB - 157 | | | |
| PCB - 167 | | | |
| PCB - 169 | | | |
| PCB - 189 | | | |
| BASE/NEUTRALS | | (ppb) | (ppb) |
| Aromatic Hydrocarbons | 8270[2] | 20[2] | 20[2] |
| Biphenyl | | | |
| Benzo(e)pyrene | | | |
| 2-6-Dimethylnaphthalene | | | |
| 1-Methylphenanthrene | | | |
| 1-Methylnaphthalene | | | |
| 2-Methylnaphthalene | | | |
| Perylene | | | |
| | | (ppb) | (ppb) |
| Phthalates | 1625C, 3540, 8250[2] | 50[2] | 20[2] |
| Dimethylphthalate | | | |
| Diethylphthalate | | | |
| Di-n-butylphthalate | | | |
| Butyl benzyl phthalate | | | |
| Bis(2-ethylhexyl) phthalate | | | |
| Di-octyl phthalate | | | |

TABLE I-1. Additional chemical constituents[1], EPA analytical methods and detection limits used for the chemical examination of proposed dredged material (continued).

[1] Chemical constituents on this optional list would be stipulated by the Corps of Engineers in cooperation with other Federal resource agencies. Any additional chemicals can be found in EPA/USACE (1995) or other EPA standard guidance.

[2] Includes all compounds listed.

[3] Includes all compounds listed unless otherwise noted.

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# APPENDIX II

# QUALITY CONTROL SUMMARY SHEETS

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**Table II-1: Completeness Checklist**

| Quality Assurance/Quality Control Questions | Yes/No? Comments? |
|---|---|
| 1. Was the report signed by the responsible applicant approved representative? | |
| 2. Were the methods for sampling, chemical and biological testing described in the Sampling and Analysis Plan (SAP) and the Laboratory QA Plan (LQAP) followed? | |
| 3. If not, were deviations documented? | |
| 4. Was the SAP approved by the New England District? | |
| 5. Did the applicant use a laboratory with a LQAP on file at the New England District? | |
| 6. Did the samples adequately represent the physical/chemical variability in the dredging area? | |
| 7. Were the correct stations sampled (include the precision of the navigation method used)? | |
| 8. Were the preservation and storage requirements in Chapter 8 of the EPA/Corps QA/QC Manual (EPA/USACE 1995) and EPA (2001d) followed? | |
| 9. Were the samples properly labeled? | |
| 10. Were all the requested data included? | |
| 11. Were the target quantitation limits (TQLs) met? | |
| 12. Were the chain-of-custody forms properly processed? | |
| 13. Were the method blanks run and were the concentration below the acceptance criteria? | |
| 14. Was the MDL study performed on each matrix (with this data submission) or within the last 6 months? | |
| 15. Were the SRM/CRM analyses within acceptance criteria? | |

| | |
|---|---|
| 16. Were the matrix spike/matrix spike duplicates run at the required frequency and was the percent recovery/RPD within the acceptance criteria? | |
| 17. Were the duplicate samples analyzed and were the RPDs within the required acceptance criteria? | |
| 18. For each analytical fraction of organic compounds, were recoveries for the internal standard within the acceptance criteria? | |
| 19. Were surrogate recoveries within the required acceptance criteria? | |
| 20. Were corrective action forms provided for all non-conforming data? | |
| 21. Were all the species-specific test conditions in Appendix V met? | |
| 22. Were the test-specific age requirements met for each test species? | |
| 23. Was the bulk physical/chemical testing performed on the sediments/composites that were biologically tested? | |
| 24. Were the mortality acceptance criteria met for the water column and sediment toxicity tests? | |
| 25. Were the test performance requirements in Table 11.3 of EPA (1994a) met? | |

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**Table II-2: Quality Control Summary** To be completed for Sediment and Tissue Matrices

Parameter: Polyaromatic Hydrocarbons (PAH) and other base-neutrals

Method Reference Number: 8270C

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Initial Calibration | Must be performed prior to the analysis of any QC sample or field sample.(<20 % RSD for each compound) | | | Retained at Lab |
| Calculation of Method Detection Limits (MDLs) | For each matrix, analyzed once per 6 month period (or with each group of field samples if MDL data have not been submitted in previous 6 months), - See Section 5.4 for MDL procedure | | | In Data Package |
| Calibration Verification (Second Source) | Once, after initial calibration (80 - 120% recovery of each compound) | | | Retained at Lab |
| Continuing Calibration | At the beginning of every 12 hour shift ( ± 15 % D) | | | Retained at Lab |
| Standard Reference Materials | Within the limits provided by vendor | | | In Data Package |
| Method Blank | No target analytes > TDL | | | In Data Package |
| Matrix Spike/Matrix Spike Duplicate (MS/MSD) | One set (MS/MSD) per group of field samples. Must contain all target analytes. (Recovery Limits 50-120%; RPD <30%) | | | In Data Package |

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Analytical Replicates | Analyze one sample in duplicate (water in triplicate) for each group of field samples (% RSD < 30) | | | In Data Package |
| Surrogate Recoveries | Calculate % recovery. (30 - 150% Rec.) | | | In Data Package |
| Internal Standard Areas | Within 50 -200% of internal standards in continuing calibration check | | | In Data Package |

* The Quality Control Acceptance Criteria are general guidelines. If alternate criteria are used, they must be documented in this table.

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**Table II-3: Quality Control Summary**

To be completed for Sediment, Tissue and Water Matrices
Parameter: Pesticides

Method Reference Number: 8081B

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Initial Calibration | Must be performed prior to the analysis of any QC sample or field sample.(<20 % RSD for each compound) | | | Retained at Lab |
| Calculation of Method Detection Limits (MDLs) | For each matrix, analyzed once per 6 month period (or with each group of field samples if MDL data have not been submitted in previous 6 months), - See Section 5.4 for MDL procedure | | | In Data Package |
| Calibration Verification (Second Source) | Once, after initial calibration. (80 - 120% recovery of each compound) | | | Retained at Lab |
| Continuing Calibration | Every 20 injections (± 15 % D) | | | Retained at Lab |
| Standard Reference Materials | Within the limits provided by vendor | | | In Data Package |
| Method Blank | No target analytes > TDL | | | In Data Package |

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Matrix Spike/Matrix Spike Duplicate (MS/MSD) | One set (MS/MSD) per group of field samples. Must contain all target analytes. (Recovery Limits 50-120%; RPD <30%) | | | In Data Package |
| Analytical Replicates | Analyze one sample in duplicate (water in triplicate) for each group of field samples (% RSD < 30) | | | In Data Package |
| Surrogate Recoveries | Calculate % recovery. (30 - 150% Rec.) | | | In Data Package |

* The Quality Control Acceptance Criteria are general guidelines. If alternate criteria are used, they must be documented in this table.

**Table II-4: Quality Control Summary** To be completed for Sediment, Tissue and Water Matrices

Parameter: Polychorinated Biphenyls (PCB congeners) Method Reference Number:8082A

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No ) | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Initial Calibration | Must be performed prior to the analysis of any QC sample or field sample.(<20 % RSD for each compound) | | | Retained at Lab |
| Calculation of Method Detection Limits (MDLs) | For each matrix, analyzed once per 6 month period (or with each group of field samples if MDL data have not been submitted in previous 6 months), - See Section 5.4 for MDL procedure | | | In Data Package |
| Calibration Verification (Second Source) | Once, after initial calibration. (80 - 120% recovery of each compound) | | | Retained at Lab |
| Continuing Calibration | Every 20 injections (± 15 % D) | | | Retained at Lab |
| Standard Reference Materials | Within the limits provided by vendor | | | In Data Package |
| Method Blank | No target analytes > TDL | | | In Data Package |
| Matrix Spike/Matrix Spike Duplicate (MS/MSD) | One set (MS/MSD) per group of field samples. Must contain all target analytes. (Recovery Limits 50-120%; RPD <30%) | | | In Data Package |

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No ) | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Analytical Replicates | Analyze one sample in duplicate (water in triplicate) for each group of field samples (% RSD < 30) | | | In Data Package |
| Surrogate Recoveries | Calculate % recovery. (30 - 150% Rec.) | | | In Data Package |

* The Quality Control Acceptance Criteria are general guidelines. If alternate criteria are used, they must be documented in this table.

**Table II-5: Quality Control Summary** To be completed for Sediments, Tissue and Water Matrices

Parameter: Metals

Method Reference Numbers: Various Reference Numbers

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Linear Range Determination for ICP | Performed Quarterly | | | Retained at Lab |
| Initial Calibration for AA, Hg | Performed Daily (Correlation Coefficient ≥0.995) | | | Retained at Lab |
| Calculation of Method Detection Limits (MDLs) | For each matrix, analyzed once per 6 month period (or with each group of field samples if MDL data have not been submitted in previous 6 months), - See Section 5.4 for MDL procedure | | | In Data Package |
| Initial Calibration Verification/ Continuing Calibration Verification | Hg: 80 -120% recovery<br>Other metals: 90 - 110% recovery | | | Retained at Lab |
| Initial Calibration Blank/ Continuing Calibration Blank | No target analytes > Instrument Detection Limit (IDL) | | | Retained at Lab |
| Standard Reference Materials | Within the limits provided by vendor | | | In Data Package |
| Method Blank | No target analytes > TDL | | | In Data Package |

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Sample Spike/ Sample Duplicate | One set per group of field samples. Must contain all target analytes. Recovery Limits (75-125%; RPD < 20% or < 35%) | | | In Data Package |
| Analytical Replicates | Analyze one sample in duplicate (water in triplicate) for each group of field samples (% RSD < 30) | | | In Data Package |

* The Quality Control Acceptance Criteria are general guidelines. If alternate criteria are used, they must be documented in this table.

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**Table II-6: Quality Control Summary** To be completed for Sediment, Tissue and Water Matrices

Parameter: Other Organic Chemicals not listed

Method Reference Number:

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Initial Calibration | Must be performed prior to the analysis of any QC sample or field sample.(<20 % RSD for each compound) | | | Retained at Lab |
| Calculation of Method Detection Limits (MDLs) | For each matrix, analyzed once per 6 month period (or with each group of field samples if MDL data have not been submitted in previous 6 months), - See Section 5.4 for MDL procedure | | | In Data Package |
| Calibration Verification (Second Source) | Once, after initial calibration (80 - 120% recovery of each compound) | | | Retained at Lab |
| Continuing Calibration | At the beginning of every 12 hour shift ( ± 15 % D) | | | Retained at Lab |
| Standard Reference Materials | Within the limits provided by vendor | | | In Data Package |
| Method Blank | No target analytes > TDL | | | In Data Package |

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Matrix Spike/Matrix Spike Duplicate (MS/MSD) | One set (MS/MSD) per group of field samples. Must contain all target analytes. (Recovery Limits 50-120%; RPD <30%) | | | In Data Package |
| Analytical Replicates | Analyze one sample in duplicate (water in triplicate) for each group of field samples (% RSD < 30) | | | In Data Package |
| Surrogate Recoveries | Calculate % recovery. (30 - 150% Rec.) | | | In Data Package |
| Internal Standard Areas | Within 50 -200% of internal standards in continuing calibration check | | | In Data Package |

* The Quality Control Acceptance Criteria are general guidelines. If alternate criteria are used, they must be documented in this table.

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**Table II-7: Quality Control Summary** To be completed for Sediments only

Parameter: Sediment Grain Size and Total Organic Carbon Analyses

Method Reference Numbers:

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Analytical Replicates | Analyze one sample in duplicate for each group of field samples (% RPD < 25%) | | | In Data Package |
| Total Organic Carbon - Standard Reference Materials | Within the limits provided by vendor | | | In Data Package |
| Total Organic Carbon Analytical Replicates | Analyze one sample in duplicate for each group of field samples (% RSD < 30) | | | In Data Package |

**Table II-8: Quality Control Summary** To be completed for Biological Testing only

Parameter: Toxicity Testing Method Reference Numbers:

| Quality Control (QC) Element | Acceptance Criteria* | Criteria Met? Yes/No | List results outside criteria (Cross-reference results table in data report) | Location of Results (Retained at Lab or in Data Package) |
|---|---|---|---|---|
| Test condition requirements for each species: Temperature, Salinity, pH, D.O., $NH_4^+$ (Total, Un-ionized) | Test conditions within the requirements specified for each species | | | In Data Package |
| Test species age | Age/health within guidelines for each species (Appendix V) | | | In Data Package |
| Bulk physical/chemical analyses (If required by the Sampling plan) | Required? If so, performed? Yes or No | | | In Data Package |
| Water column toxicity test:<br>Control mortality<br>Control abnormality | <br>≤ 10% mean<br>≤ 30% mussel/oyster; ≤ 40% clam larvae | | | In Data Package |
| Sediment toxicity test:<br>Control mortality<br>Compliance with applicable test acceptability requirements in Table 11.3 (EPA 1994a) | <br>≤ 10% mean (no chamber > 20%)<br>See EPA (1994a) Section 9; Table 11.3 | | | In Data Package |

* The Quality Control Acceptance Criteria are general guidelines. If alternate criteria are used, they must be documented in this table.

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# APPENDIX III

# FORMS FOR ATTERBERG LIMITS

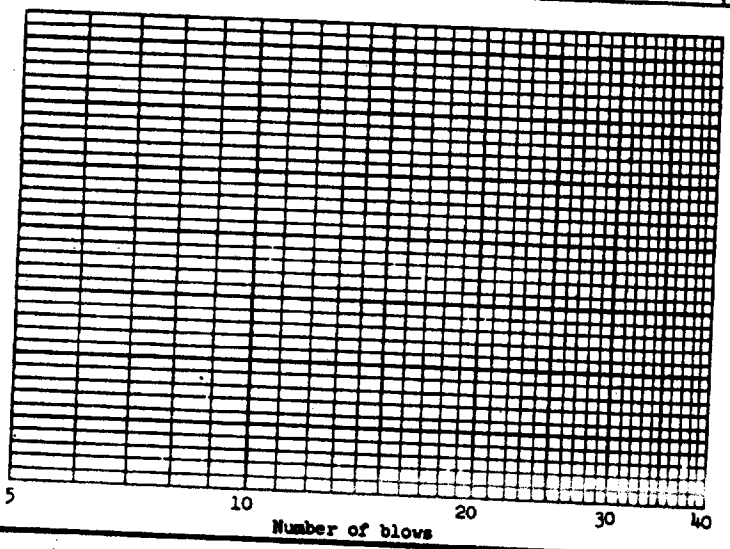
## LIQUID AND PLASTIC LIMIT TESTS

Project _____ Date _____

Boring No. _____ Sample No. _____

### LIQUID LIMIT

| Run No. | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| Tare No. | | | | | | | |
| Weight in grams: Tare plus wet soil | | | | | | | |
| Tare plus dry soil | | | | | | | |
| Water | $W_w$ | | | | | | |
| Tare | | | | | | | |
| Dry soil | $W_s$ | | | | | | |
| Water content | w | | | | | | |
| Number of blows | | | | | | | |




LL _____

PL _____

PI _____

Symbol from plasticity chart _____

### PLASTIC LIMIT

| Run No. | | 1 | 2 | 3 | 4 | 5 | Natural Water Content |
|---|---|---|---|---|---|---|---|
| Tare No. | | | | | | | |
| Weight in grams: Tare plus wet soil | | | | | | | |
| Tare plus dry soil | | | | | | | |
| Water | $W_w$ | | | | | | |
| Tare | | | | | | | |
| Dry soil | $W_s$ | | | | | | |
| Water content | w | | | | | | |
| Plastic limit | | | | | | | |

Remarks _____

Technician _____ Computed by _____ Checked by _____

ENG FORM 1 JUN 55 3838

PLATE III-1

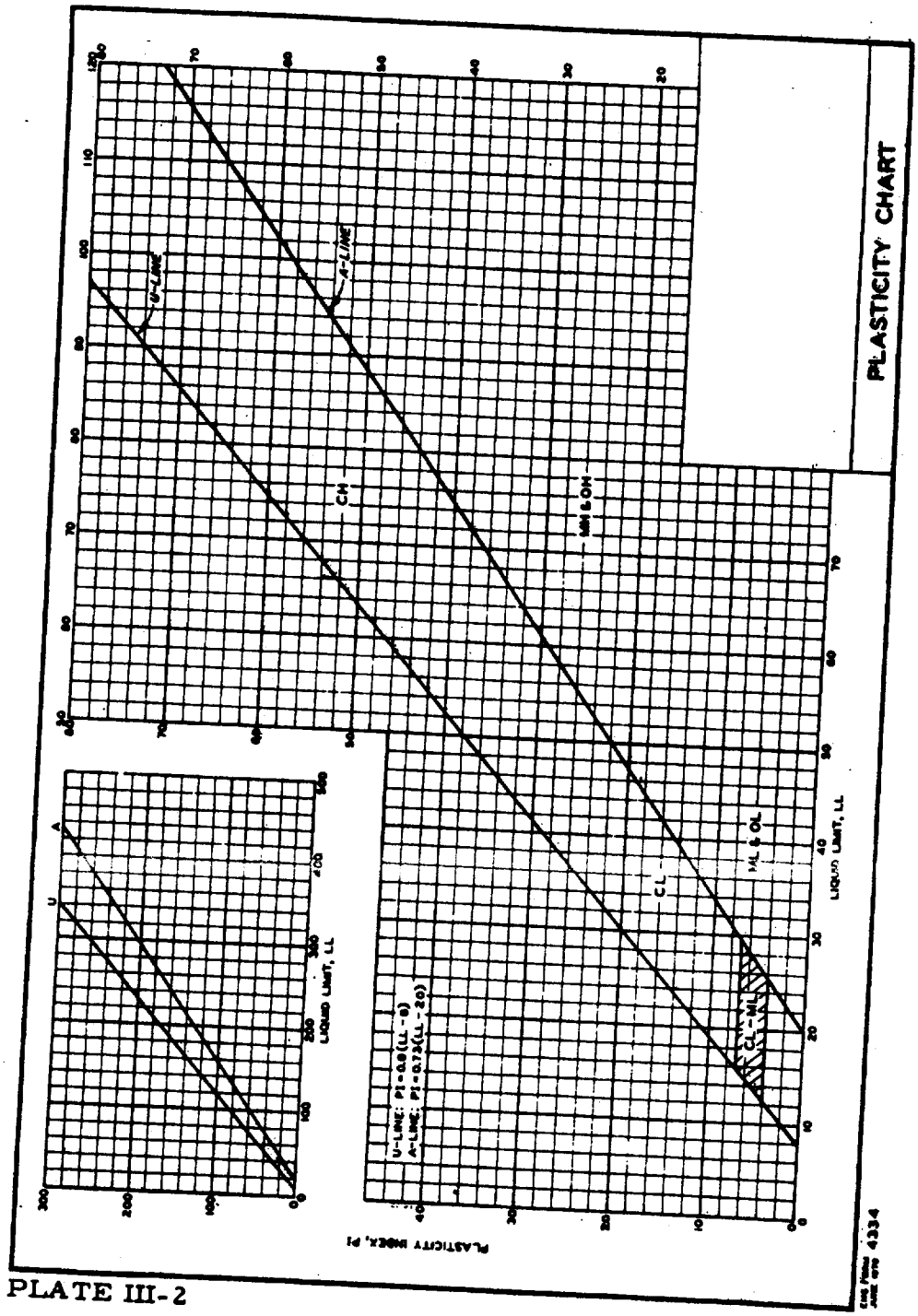

PLASTICITY CHART
A-LINE
U-LINE
CH
MH & OH
CL
ML & OL
CL - ML
LIQUID LIMIT, LL
PLASTICITY INDEX, PI
U-LINE: PI = 0.9(LL - 8)
A-LINE: PI = 0.73(LL - 20)

PLATE III-2

9/5/02

# APPENDIX IV

# PROCEDURES FOR COLLECTION OF LARGE VOLUME WATER SAMPLES

**POINT OF CONTACT:**

Richard McKinney, Chemistry Group
Atlantic Ecology Division
US Environmental Protection Agency
27 Tarzwell Dr.
Narragansett, RI 02882

## 1.0 OBJECTIVES

The objective of this document is to describe the recommended field use of the high volume water sampling apparatus. This apparatus concentrates particulate material on a glass fiber filter and extracts dissolved non-ionic organic contaminants polychlorinated biphenyls and chlorinated pesticides on polyurethane foam plugs from a large (10-20 L) water sample. Also included in this LOP is information that may be useful in trouble shooting problems encountered.

## 2.0 MATERIALS AND EQUIPMENT

- High volume pump
- Stainless steel coated hoses
- Filter housing
- Foam plug housings (loaded with extracted plugs)
- Generator
- Pre-combusted Type A/E glass fiber filters 293 mm
- Acetone rinsed stainless steel cans with tops
- TWO 18 L containers with DI water
- Labeling tape
- Lab marker
- Lab notebook
- Gloves (field gloves and plastic lab gloves)
- Large ziplock bags

- Teflon tape
- Duct tape
- Cooler with ice
- Forceps
- Spatulas
- Filter housing wrench
- Crescent wrenches 1 1/4" (2), 11/16", 1", 7/8"
- Two large adjustable wrenches
- One hammer

## 3.0 PROCEDURE

### 3.1 Preparation

3.1.1. If the pump, hoses, filter housing, and foam plug housings have not been recently used, they should be cleaned well with Alconox and tap water. If possible, the pump should be set up in the lab and tap water circulated through it. Any parts of the apparatus that can be should be thoroughly rinsed with DI water prior to use.

Note: The stainless steel covering the hoses is frayed in some places. It is advisable to wear work gloves whenever manipulating them to avoid cutting your hands.

3.1.2. Filters should be individually wrapped in clean aluminum foil and combusted in a 450°C oven for 6 hours. After the filters have been combusted it is extremely important that they not be bent, twisted or disturbed in any way. They should be taken out of the oven and immediately placed in a covered container in which they can remain until it is time for them to be used. There should be one filter for each sample, one for each field blank and at least three extra.

3.1.3. Filter containers (stainless steel cans with tops) should be washed, rinsed with DI water and cleaned with acetone.

3.1.4. The procedure for the preparation for the foam plugs is included in the LOP for the analysis of dissolved organics using foam plugs (AED LOP 2.03.018). The housings should be wrapped in clean aluminum foil for transport to the field.

3.2 Field Use

3.2.1. The pump will float when placed in the water however, a safety line should be tied from it to the boat.

3.2.2. Pass the intake hose through the water filling it completely with water. This is necessary to prime the pump. Attach the intake hose to the pump.

3.2.3. Attach the outflow hose to the pump.

3.2.4. Start the generator and start the pump. There should be a strong flow of water out of the outflow hose.

3.2.5. Once the pump is primed, it may be turned off as long as the operators are careful not to allow air to enter the device. At this time, open the filter housing and very carefully place one filter on the screened platform. Hand tighten the screws and then completely tighten them with the filter housing wrench.

3.2.6. Attach the hose from the bottom of the filter housing to the top of the foam plug housing.

3.2.7. To take a seawater sample, place the end of the intake hose in the water making sure not to introduce any air into the system. Start the pump for 5 seconds. Stop the pump. Attach the hose from the outflow of the pump to the top of the filter housing. Open the air bleed valve on the top of the filter housing. Start the pump. Shut the air bleed valve once the air stops coming out (approximately 5 seconds). There should be a trickle of water coming out of the foam plug. A second hose may be attached to the outflow of the foam plug housing and the end placed in the empty 18L DI water container. This will make it possible to measure the volume of water sampled.

3.2.8. Pump 18 liters or other amount of water through the apparatus. Turn off the pump. If the apparatus has not been used recently or was last used in a contaminated area it would be advisable to take another field blank before sampling the seawater.

3.2.9. Open the air bleed valve on the filter housing. Unscrew the housing top and carefully remove the top. Examine the filter to see if it is intact. If it is, use the spatulas to fold the filter and place it in the stainless steel can. Label the can.

3.2.10. Replace the ends of the foam plug housing. Label the housing and wrap it in aluminum foil. Place the filter and foam plug on ice in the cooler.

## 4.0 QA/QC

The primary concern at the point of collection of samples for further analysis is to verify that the system is free from initial contamination and that no cross contamination occurs between sample locations. This is accomplished by the collection of field blanks as necessary.

### 4.1 Field Blanks

4.1.1. To take the field blank, place the end of the intake hose in the DI water container making sure not to introduce any air into the system. Start the pump for 5 seconds. Stop the pump. Attach the hose from the outflow of the pump to the top of the filter housing. Open the air bleed valve on the top of the filter housing. Start the pump. Shut the air bleed valve once the air stops coming out (approximately 5 seconds). There should be a trickle of water coming out of the foam plug.

4.1.2. Pump as much of the 18 liters of DI water as you can through the apparatus without getting any air in the system. This should take approximately 10-15 minutes. Turn off the pump. If the apparatus has not been used recently or was last used in a contaminated area it would be advisable to take another field blank before sampling the seawater. Place the intake hose in the second 18 liters of DI water before changing the

foam plug and the filter. If not taking a second field blank, the intake hose may be place back into the seawater.

4.1.3. Open the air bleed valve on the filter housing. Unscrew the housing top and carefully remove the top. Examine the filter to see if it is intact. If it is, use the spatulas to fold the filter and place it in the stainless steel can. Label the can.

4.1.4. Replace the ends of the foam plug housing. Label the housing and wrap it in aluminum foil. Place the filter and foam plug on ice in the cooler.

## 5.0 TROUBLE SHOOTING

5.1. *Pump is on, no water flow* - The pump has not been primed properly. Purge the intake hose of air and reattach. Hold the outflow hose and the foam plug lower in the boat.

5.2. *The filter housing leaks* - Wipe standing water off of the top of the housing. Use the filter wrench to tighten the screws.

5.3. *Leaks occur at hosing attachments* - Use teflon tape to wrap the male connectors prior to use.

5.4. *Filters break* - Experience has shown the breaking filters usually are the result of rough handling. Place the next filter on and make sure to shield the housing and filter from the wind while putting the filter on.

## 6.0 REFERENCES

None.

9/5/02

# APPENDIX V

# SEA URCHIN LARVAL TOXICITY TEST PROCEDURE

## POINT OF CONTACT:

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Diane Nacci
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## 1. OBJECTIVES

The purpose of the sea urchin larval development test is to determine the effects of effluents and water samples on survival, growth, and development of larvae of the sea urchin, Arbacia punctulata.

## 2. MATERIALS AND EQUIPMENT

- Facilities for holding and acclimating test organisms.
- Laboratory sea urchin culture unit -- See culturing LOP. To test effluent or receiving water toxicity, sufficient eggs and sperm must be available.
- Environmental chamber or equivalent facility with temperature control (20±1°C) for controlling temperature during exposure.
- Water purification system -- Millipore Super-Q, Deionized water (DI) or equivalent. - Balance -- Analytical, capable of accurately weighing to 0.0001 g.
- Reference weights, Class S -- for checking performance of balance.
- Air pump -- for supplying air.
- Air lines, and air stones -- for aerating water containing adults.
- Vacuum suction device -- for washing eggs.
- pH and DO meters -- for routine physical and chemical measurements. Unless the test is being conducted to specifically measure the effect of one of the parameters, portable, field-grade instruments are acceptable.
- Transformer, 10-12 Volt, with steel electrodes -- for stimulating release of eggs and sperm.
- Centrifuge, bench-top, slant-head, variable speed -- for washing eggs.
- Fume hood -- to protect the analyst from formaldehyde fumes.
- Dissecting microscope -- for counting diluted egg stock.

- Compound microscope -- for examining and counting sperm cells and fertilized eggs.
- Compound microscope with CCD digital camera and low powered objectives (2-10x magnification) -- for use with image analyzer (quantification of growth endpoint).
- Cambridge Instruments Quantimet 520 image analyzer with IBM PC/AT (or equivalent) and video display -- for quantification of growth endpoint.
- Sedgwick-Rafter counting chamber -- for counting egg stock and final examination of larvae.
- Hemacytometer, Neubauer -- for counting sperm.
- Count register, 2-place -- for recording sperm and egg counts.
- Refractometer -- for determining salinity.
-Thermometers, glass or electronic, laboratory grade -- for measuring water temperatures.
-Thermometers, bulb-thermograph or electronic-chart type -- for continuously recording temperature.
- Ice bucket, covered -- for maintaining live sperm.
- Centrifuge tubes, conical, 15 mL -- for washing eggs.
- Cylindrical glass vessel, 8-cm diameter -- for maintaining dispersed egg suspension.
- Beakers -- at least six Class A, borosilicate glass or non-toxic plasticware, 1000 mL for making test solutions.
- Glass dishes, flat bottomed, 20-cm diameter -- for holding adult urchins during gamete collection.
- Wash bottles -- for deionized water, for rinsing small glassware and instrument electrodes and probes.
- Volumetric flasks and graduated cylinders -- Class A, borosilicate glass or non-toxic plastic labware, 10-1000 mL for making test solutions.
- Syringes, 1-mL, and 10-mL, with 18 gauge, blunt-tipped needles (tips cut off) -- for collecting sperm and eggs.
- Pipets, volumetric -- Class A, 1-100 mL.
- Pipets, automatic -- adjustable, 1-100 mL.
- Pipets, serological -- 1-10 mL, graduated.
- Pipet bulbs and fillers -- PROPIPET$^{R}$, or equivalent.
- Tape, colored -- for labelling tubes.
- Markers, water-proof -- for marking containers, etc.
- Sea Urchins (approximately 12 of each sex).
- Scintillation vials, 20 mL, disposable -- to prepare test concentrations.
- Parafilm -- to cover tubes and vessels containing test materials.
- Gloves, lab coat, disposable -- for personal protection from contamination.
- Safety glasses.
- Data sheets (one set per test) -- for data recording (Figure 1).

- Acetic acid, 10%, reagent grade, in sea water -- for preparing killed sperm dilutions.
- Formalin, 10% in seawater -- for preserving eggs.
- pH buffers 4, 7, and 10 (or as per instructions of instrument manufacturer) for standards and calibration check.
- Reagent water -- defined as distilled or deionized water that does not contain substances which are toxic to the test organisms.
- Effluent, surface water, and dilution water.
- Saline test and dilution water -- The salinity of the test water must be 30‰. The salinity should vary by no more than ± 2‰ among the replicates.

## 3. PROCEDURE

A. Test Solutions

1. Samples are used directly as collected when sample salinity is between 28 and 32 parts per thousand. If samples do not require salinity adjustment natural seawater is used in all washing and diluting steps. Local uncontaminated water may be used as an additional control.

2. If salinity adjustment is required, prepare 3 L of control water at 30‰ using hypersaline brine (see Brine LOP). This water is used in all washing and diluting steps and as control water in the test. Natural sea water and uncontaminated local waters may be used as additional controls.

3. Effluent/receiving water samples are adjusted to salinity of 30 $^{O}$/oo.

4. The selection of the effluent test concentrations should be based on the objectives of the study. A dilution factor of 0.5 is used with this procedure, starting with a high concentration of 70% effluent (for freshwater effluents). If the effluent is known or suspected to be highly toxic, a lower range of effluent concentrations should be used.

5. Three replicates are prepared for each test concentration, using 10 mL of solution in disposable liquid scintillation vials. A 50% (0.5) concentration series can be preparedby serially diluting test concentrations with control water.

6. All test samples are equilibrated at 20 ± 1°C before addition of sperm.

B. Collection and Preparation of Gametes for the Test

1. Select four females and place in shallow bowls, barely covering the shell with seawater. Stimulate the release of eggs by touching the test with electrodes from the transformer. Collect about 3 mL of eggs from each female using a syringe with a blunted needle. Remove the needle from the syringe before adding the eggs to a 15 mL conical centrifuge tube. Pool the eggs. The egg stock may be held at room temperature for several hours before use. Note: The egg suspension may be prepared during the l-h sperm exposure.

2. Select four males and place in shallow bowls, barely covering the animals with seawater. Stimulate the release of sperm by touching the shell with steel electrodes connected to a 12 V transformer (about 30 seconds each time). Collect the sperm (about 0.25 mL) from each male, using a 1 mL disposable syringe fitted with an 18-gauge, blunt-tipped needle. Maintain the syringe containing pooled sperm sample on ice. The sperm must be used in a toxicity test within 1 h of collection.

3. Using control water, dilute the pooled sperm sample to a concentration of about 5 X $10^7$ sperm/mL (SPM). Estimate the sperm concentration as described below:

   a. Make a sperm dilutions of 1:50, 1:100, 1:200, and 1:400, using 30‰ seawater, as follows:

      1. Add 400 uL of collected sperm to 20 mL of sea water in Vial A. Mix by gentle pipetting using a 5-mL pipetter.

      2. Add 10 mL of sperm suspension from Vial A to 10 mL of seawater in Vial B. Mix by gentle pipetting using a 5-mL pipetter.

      3. Add 10 mL of sperm suspension from Vial B to 10 mL of seawater in Vial C. Mix by gentle pipetting using a 5-mL pipetter.

      4. Add 10 mL of sperm suspension from Vial C to 10 mL of seawater in Vial D. Mix by gentle pipetting using a 5-mL pipetter.

5. Discard 10 mL from Vial D. (The volume of all suspensions is 10 mL).

b. Make a 1:2000 killed sperm suspension and determine the SPM.

1. Add 10 mL 10% acetic acid in seawater to Vial C. Cap Vial C and mix by inversion.

2. Add 1 mL of killed sperm from Vial C to 4 mL of seawater in Vial E. Mix by gentle pipetting with a 5-mL pipetter.

3. Add sperm from Vial E to both sides of the Neubauer hemacytometer. Let the sperm settle 15 min.

4. Count the number of sperm in the central 400 squares on both sides of the hemacytometer using a compound microscope (400X). Average the counts from the two sides.

5. SPM in Vial E = $10^4$ x average count.

c. Calculate the SPM in all other suspensions using the SPM in Vial E above:

SPM in Vial A = 40 x SPM in Vial E
SPM in Vial B = 20 x SPM in Vial E
SPM in Vial D = 5 x SPM in Vial E
SPM in original sperm sample = 2000 x SPM in Vial E

d. Dilute the sperm suspension with a concentration greater than 5 x $10^7$ SPM to 5 x $10^7$ SPM.

Actual SPM/(5 x $10^7$) = dilution factor (DF)

[(DF) x 10] - 10 = mL of seawater to add to vial.

4. Wash the pooled eggs three times using control water with gentle centrifugation (500xg or the lowest possible setting) for 3 min using a tabletop centrifuge). If the wash water becomes red, the eggs have lysed and must be discarded.

a. Dilute the egg stock, using control water, to about 2000 ± 200 eggs/mL.

1. Remove the final wash water from the eggs and transfer the washed eggs (by refilling the centrifuge tube with control water and repeatedly inverting to resuspend the eggs) to a beaker containing a small amount (about 50 mL) of control water. Add control water to bring the eggs to a volume of 200 mL ("egg stock").

2. Mix the egg stock using gentle aeration. Cut the point from a pipet tip and transfer l mL of eggs from the egg stock to a vial containing 9 mL of control water. (This vial contains an egg suspension diluted l:l0 from egg stock).

3. Mix the contents of the vial using gentle pipetting. Cut the point from a pipet tip and transfer 1 mL of eggs from the vial to a Sedgwick-Rafter counting chamber. Count all eggs in the chamber using a dissecting microscope at l0X ("egg count").

4. Calculate the concentration of eggs in the stock. Eggs/mL = l0x (egg count). Dilute the egg stock to 2000 eggs mL by the formula below.

b. If the egg count is equal to or greater than 200:

(egg count) - 200 = volume (mL) of control water to add to egg stock

c. If the egg count is less than 200, allow the eggs to settle and remove enough control water to concentrate the eggs to greater than 200, repeat the count, and dilute the egg stock as above. 100 mL of egg stock are required to perform this test.

d. Transfer 1 mL of the diluted egg stock to a vial containing 9 mL of control water. Mix well, then transfer 1 mL from the vial to a Sedgwick-Rafter counting chamber. Count all eggs using a dissecting microscope. Confirm that the final egg count = 200/mL ± 20.

5. Mix the egg stock well, subsample 100 mL, and place the subsample in a clean beaker. Add 10 mL of the proper sperm dilution to the beaker and mix well. This will result in a egg:sperm ratio of 1:2500, which should allow acceptable egg fertilization 1 hr after sperm addition.

C. Start of the Test

1. Mix the diluted embryo suspension (2000 embryos/mL), using gentle aeration. Add 1 mL of diluted egg suspension to each test vial using a wide mouth pipet tip. Incubate covered for 48 hours $20 \pm 1^{O}C$.

D. Termination of the Test

1. Terminate the test and preserve the samples by adding 2 mL of 10% formalin in seawater to each vial.

2. Vials may be evaluated immediately or capped and stored for as long as one week before being evaluated.

3. Each vial is thoroughly mixed and a 1 mL aliquot added to a Sedgwick-Rafter counting chamber for microscopic observation and image analysis. The total number of larvae and of appropriately developed larvae (pluteii) are counted to determine survival and development for each treatment. Fifty larvae per replicate are also observed using the image analysis system and measured for maximum length, total area, and shape (a function relating observed shape to that of a circle).

## 4. QA/QC

A. STATISTICAL ANALYSIS AND DATA USAGE

1. Tabulate and summarize the data.

2. An estimate of the effluent concentration which would cause a 50% toxic effect (EC50) for each parameter is calculated using Trimmed Spearman-Karber analysis (Hamilton, Russo, and Thurston, 1977). One-way analysis of variance (ANOVA) followed by Dunnett's Procedure (Dunnett, 1955) is used to compare single

treatments to the control in order to estimate no effect and least effect concentrations (NOEC and LOEC values).

3. Data are used along with other toxicity tests in assessing the toxicity of an effluent or receiving water.

## 5. TROUBLE SHOOTING

1. Toxic substances may be introduced by contaminants in dilution water, glassware, sample hardware, and testing equipment.

## 6. REFERENCES

Dunnett, C.W. 1955. A multiple comparisons procedure for comparing several treatments with a control. JASA 50:1096-1101.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11(7):714-719.

US EPA. 1988. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Weber, C.I., et al (eds). EPA Office of Research and Development EPA-600/4-87/028 (May 1988).

9/5/02

# APPENDIX VI

# SPECIES-SPECIFIC TESTING CONDITIONS

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSID SHRIMP, *Mysidopsis bahia, M. bigelowi, M. almyra, Neomysis americana, Holmesimysis costata*, ACUTE TOXICITY WATER COLUMN TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal |
| 2. Test duration: | 96 h |
| 3. Temperature: | 20±1°C: or 25±1°C for<br>*Mysidopsis bahia*<br>*Mysidopsis bigelowi*<br>*Mysidopsis almyra*<br>20±1°C for<br>*Neomysis americana*<br>12±1°C for<br>*Holmesimysis costata* |
| 4. Salinity: | 25-30 ‰ ±10% except for *Holmesimysis costata* which is to be 32-34 ‰ ±10% |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m²/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size: | 250 mL minimum |
| 9. Test solution volume: | 200 mL minimum |
| 10. Renewal of test solutions: | None |
| 11. Age of test organisms: | 1 - 5 d; 24 h range in age |
| 12. No. organisms per test chamber: | 10 minimum |
| 13. No. replicate chambers per concentration: | 5 minimum |
| 14. No. organisms per concentration: | 50 minimum |
| 15. Feeding regime: | *Artemia* nauplii are made available while holding prior to the test; feed 0.2 mL of concentrated suspension of *Artemia* nauplii ≤24 h old, daily (approximately 100 nauplii per mysid) |
| 16. Test chamber cleaning: | None |
| 17. Test solution aeration: | If needed to maintain DO> 40% for:<br>*Mysidopsis bahia*<br>*Mysidopsis bigelowi*<br>*Mysidopsis almyra*<br>*Neomysis americana*<br>and DO> 60% saturation for:<br>*Holmesimysis costata*<br>(< 100 bubbles/min.) |
| 18. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water |
| 19. Test concentrations: | Three concentrations for site sediment, and control water |
| 20. Dilution series: | 100%, 50%, 10% |
| 21. Endpoint: | Survival |

| | |
|---|---|
| 22. Sampling and sample holding requirements: | <8 wk (sediment); elutriates are to be used within 24 h of preparation |
| 23. Sample volume required: | 1 L per site |
| 24. Test acceptability criterion: | ≥ 90% survival in controls |

**REFERENCE:**

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SHEEPSHEAD MINNOW, *Cyprinodon variegatus*, INLAND SILVERSIDE, *Menidia beryllina*, ATLANTIC SILVERSIDE, *M. menidia*, TIDEWATER SILVERSIDE, *M. peninsulae*, ACUTE TOXICITY WATER COLUMN TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal |
| 2. Test duration: | 96 h |
| 3. Temperature: | 20 or 25±1°C |
| 4. Salinity: | Sheepshead minnow: 5-30 ‰ ± 10%<br>Silversides: 5-32 ‰ ± 10% |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m$^2$/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size: | 250 mL minimum |
| 9. Test solution volume: | 200 mL minimum |
| 10. Renewal of test solutions: | None |
| 11. Age of test organisms: | Sheepshead minnow: 1 - 14 d; 24-h range in age<br>Silversides: 9 - 14 d; 24-h range in age |
| 12. No. organisms per test chamber: | 10 minimum |
| 13. No. replicate chambers per concentration: | 5 minimum |
| 14. No. organisms per concentration: | 50 minimum |
| 15. Feeding regime: | *Artemia* nauplii are made available while holding prior to the test; add 0.2 mL *Artemia* nauplii concentrate at 48 h |
| 16. Test chamber cleaning: | None |
| 17. Test solution aeration: | If needed to maintain DO> 40% saturation (< 100 bubbles/min.) |
| 18. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millpore MILLI-Q® or equivalent or deionized water |
| 19. Test concentrations: | Three concentrations for site sediment, and control water |
| 20. Dilution series: | 100%, 50%, 10% |
| 21. Endpoint: | Survival |
| 22. Sampling and sample holding requirements: | <8 wk (sediment); elutriates are to be used within 24 h of preparation |
| 23. Sample volume required: | 4 L per site |
| 24. Test acceptability criterion: | ≥ 90% survival in controls |

**REFERENCE:**

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

**REFERENCE**:

Adapted in part from the *Menidia* sp. protocol published in:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90-027.

and from EPA in-house expertise, ERL-Narragansett, RI.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR OYSTER, *Crassostrea virginica*, AND MUSSEL, *Mytilus edulis*, ACUTE TOXICITY WATER COLUMN TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal |
| 2. Test duration: | 48 h |
| 3. Temperature: | 25±1° C for *Crassostrea virginica*<br>16±1° C for *Mytilus edulis* |
| 4. Salinity: | 18-32± 1 ‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m²/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size:* | 1 L |
| 9. Test solution volume:* | 500 mL |
| 10. Renewal of test solutions: | None |
| 11. Age of test organisms: | Larvae less than 4 h old |
| 12. No. organisms per test chamber: | 7,500 - 15,000 |
| 13. No. replicate chambers per concentration: | 5 minimum |
| 14. No. organisms per concentration: | 22,500 - 45,000 |
| 15. Feeding regime: | None |
| 16. Test chamber cleaning: | None |
| 17. Test solution aeration: | None |
| 18. Dilution water:* | Natural seawater or modified GP2, Forty Fathoms®, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water |
| 19. Test concentrations: | Three concentrations for site sediment, and control water |
| 20. Dilution series: | None |
| 21. Endpoint: | Shell development to hinged, D-shaped prodissoconch I larva |
| 22. Sampling and sample | <8 wk (sediment); elutriates are to be used within 24 h of preparation |
| 23. Sample volume required: | 1 L per site |
| 24. Test acceptability * criterion: | ≥ 70% or greater survival and ≥ 70% shell development in controls |

* - Protocol dependent

**REFERENCE:**

ASTM. 1989. E 724-89. Standard guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SEA URCHINS, *Strongylocentrotus* sp., *Lytechinus pictus*, AND SAND DOLLAR, *Dendraster* sp., ACUTE TOXICITY WATER COLUMN TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal |
| 2. Test duration: | 48 h |
| 3. Temperature: | 12°C |
| 4. Salinity: | 30-32 ‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m$^2$/s (50-100 ft-c) |
| 7. Photoperiod: | Not essential |
| 8. Test chamber size: | 20 mL minimum |
| 9. Test solution volume: | 10 mL minimum |
| 10. Renewal of test solutions: | None |
| 11. Age of test organisms: | ≤ 1 h embryos |
| 12. No. organisms per test chamber: | 2000 |
| 13. No. replicate chambers per concentration: | 3 minimum |
| 14. No. organisms per concentration: | 6000 minimum |
| 15. Feeding regime: | None |
| 16. Test chamber cleaning: | None |
| 17. Test solution aeration: | None |
| 18. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water and 3x brine to maintain constant salinity across tests |
| 19. Test concentrations: | Three concentrations for site sediment, and control water |
| 20. Dilution series: | 100%, 50%, 10% |
| 21. Endpoint: | Survival, Embryo Development |
| 22. Sampling and sample holding requirements: | <8 wk (sediment); elutriates are to be used within 24 h of preparation |
| 23. Sample volume required: | 1 L per site |
| 24. Test acceptability criterion: | ≥ 70% survival and ≥ 70% normal embryo development in controls |

**REFERENCE:**

USEPA. 1990. Conducting the Sea Urchin Larval Development Test. ERL-Narragansett Standard Operating Procedure 1.03.007.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Ampelisca abdita*, ACUTE TOXICITY SEDIMENT TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal* |
| 2. Test duration: | 10 d |
| 3. Temperature: | 20°C |
| 4. Salinity: | 20 to 35 ‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m$^2$/s (50-100 ft-c) |
| 7. Photoperiod: | Continuous Light |
| 8. Test chamber size: | 1 L |
| 9. Test solution volume: | Vol. to 950 mL |
| 10. Sediment depth: | 4 cm minimum |
| 11. Renewal of test solutions: | None* |
| 12. Age of test organisms: | Immature amphipods, or mature females only |
| 13. No. of organisms per test chamber: | 20 |
| 14. No. replicate chambers per sediment: | 5 |
| 15. No. organisms per sediment: | 100 to 150 |
| 16. Feeding regime: | None |
| 17. Test chamber cleaning: | None |
| 18. Test solution aeration: | Trickle-flow (< 100 bubbles/min.) |
| 19. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water |
| 20. Test concentrations: | Site sediment, a reference sediment and a control sediment |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Survival |
| 23. Sampling and sample holding requirements: | <8 wk |
| 24. Sample volume required: | 2 L |
| 25. Test acceptability criterion: | ≥ 90% survival in controls |

**REFERENCE:**

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Leptocheirus plumulosus*, ACUTE TOXICITY SEDIMENT TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal* |
| 2. Test duration: | 10 d |
| 3. Temperature: | 20-25°C |
| 4. Salinity: | 20 ‰ (range 2 - 32 ‰) |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m$^2$/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size: | 1 L |
| 9. Test solution volume | Vol. to 950 mL |
| 10. Sediment depth: | 2 cm minimum |
| 11. Renewal of test solutions: | None* |
| 12. Age of test organisms: | Mature 3 - 5 mm mixed sexes |
| 13. No. of organisms per test chamber: | 20 |
| 14. No. replicate chambers per sediment: | 5 |
| 15. No. organisms per sediment: | 100 |
| 16. Feeding regime: | None |
| 17. Test chamber cleaning: | None |
| 18. Test solution aeration: | Trickle-flow (< 100 bubbles/min.) |
| 19. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water |
| 20. Test concentrations: | N/A |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Survival |
| 23. Sampling and sample holding requirements: | <8 wk |
| 24. Sample volume required: | 2 L |
| 25. Test acceptability criterion: | ≥ 90% survival in controls |

**REFERENCE:**

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Schlekat, C.E., B.E. McGee and E. Reinharz. 1992. Testing sediment toxicity in Chesapeake Bay using the amphipod *Leptocheirus plumulosus*: an evaluation. Environ. Toxicol. Chem. 11: 225-236.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Eohaustorius estuarius*, ACUTE TOXICITY SEDIMENT TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal* |
| 2. Test duration: | 10 d |
| 3. Temperature: | 15±3°C |
| 4. Salinity: | 2 to ≤28 ‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m²/s (50-100 ft-c) |
| 7. Photoperiod: | Continuous Light |
| 8. Test chamber size: | 1 L |
| 9. Test solution volume: | Vol. to 950 mL |
| 10. Sediment depth: | 2 cm minimum |
| 11. Renewal of test solutions: | None* |
| 12. Age of test organisms: | Mature amphipods, 3 -5 mm, mixed sexes |
| 13. No. of organisms per test chamber: | 20 |
| 14. No. replicate chambers per sediment: | 5 |
| 15. No. organisms per sediment: | 100 |
| 16. Feeding regime: | None |
| 17. Test chamber cleaning: | None |
| 18. Test solution aeration: | Trickle-flow (< 100 bubbles/min.) |
| 19. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water |
| 20. Test concentrations: | Site sediment, a reference sediment and a control sediment |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Survival |
| 23. Sampling and sample holding requirements: | <8 wk |
| 24. Sample volume required: | 2 L |
| 25. Test acceptability criterion: | ≥ 90% survival in controls |

**REFERENCE:**

ASTM. 1994. E1367-92. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE FRESHWATER AMPHIPOD, *Hyalella azteca*, ACUTE TOXICITY SEDIMENT TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal* |
| 2. Test duration: | 10 d |
| 3. Temperature: | 20 - 25°C |
| 4. Salinity | 0-15 ‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size: | 300 mL minimum |
| 9. Test solution volume: | Variable, depending on test type |
| 10. Sediment depth: | 2 cm minimum |
| 11. Renewal of test solutions: | None* |
| 12. Age of test organisms: | 7 - 14 d |
| 13. No. organisms per test chamber: | 10 minimum |
| 14. No. replicate chambers per sediment: | 5 minimum |
| 15. No. organisms per sediment: | 50 minimum |
| 16. Feeding regime: | Variable (None, Tetrafin, YCT*, rabbit chow, maple leaves) |
| 17. Test chamber cleaning: | None |
| 18. Test solution aeration: | Trickle-flow (<100 bubbles/min.) |
| 19. Dilution water: | Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness |
| 20. Test concentrations: | Site sediment, a reference sediment and a control sediment |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Survival |
| 23. Sampling and sample holding requirements: | <8 wk |
| 24. Sample volume required: | 2 L |
| 25. Test acceptability criterion: | ≥ 80% survival in controls |

* Slurry of Yeast, Cereal flakes, Trout chow

**REFERENCES:**

ASTM. 1994. Method E1383-94. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

USEPA. 1994. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-94/024. U.S. Environmental Protection Agency, Duluth, MN.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSID SHRIMP, *Mysidopsis bahia, M. bigelowi, M. almyra, Neomysis americana, Holmesimysis costata*, ACUTE TOXICITY SEDIMENT TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal* |
| 2. Test duration: | 10 d |
| 3. Temperature: | 20±1°C: or 25±1°C for<br>*Mysidopsis bahia*<br>*Mysidopsis bigelowi*<br>*Mysidopsis almyra*<br>20±1°C for<br>*Neomysis americana*<br>12±1°C for<br>*Holmesimysis costata* |
| 4. Salinity: | 25-30 ‰ ±10% except for *Holmesimysis costata* which is to be 32-34 ‰ ±10% |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m²/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size: | 250 mL (minimum) |
| 9. Test solution volume: | 200 mL (minimum) |
| 10. Sediment depth: | 2 cm minimum |
| 11. Renewal of test solutions: | None* |
| 12. Age of test organisms: | 1 - 5 d; 24 h range in age |
| 13. No. organisms per test chamber: | 10 minimum |
| 14. No. replicate chambers per concentration: | 5 minimum |
| 15. No. organisms per concentration: | 50 minimum |
| 16. Feeding regime: | *Artemia* nauplii are made available while holding prior to, but not during, the test; feed 0.2 mL of concentrated suspension of *Artemia* nauplii ≤24 h old, daily (approximately 100 nauplii per mysid) |
| 17. Test chamber cleaning: | None |
| 18. Test solution aeration: | If needed to maintain DO> 40% saturation for:<br>*Mysidopsis bahia*<br>*Mysidopsis bigelowi*<br>*Mysidopsis almyra*<br>*Neomysis americana*<br>and DO> 60% saturation for:<br>*Holmesimysis costata*<br>(< 100 bubbles/min.) |
| 19. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water |
| 20. Test concentrations: | Site sediment, a reference sediment and a control sediment |
| 21. Dilution series: | N/A |

| | |
|---|---|
| 22. Endpoint: | Survival |
| 23. Sampling and sample holding requirements: | <8 wk |
| 24. Sample volume required: | 1 L |
| 25. Test acceptability criterion: | ≥ 90% survival in controls |

**REFERENCE:**

Modified from:

USEPA. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 4th Ed. EPA/600/4-90/027.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.

**SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MIDGES, *Chironomus tentans* AND *C. riparius*, ACUTE TOXICITY SEDIMENT TESTS**

| | |
|---|---|
| 1. Test type: | Static Non-renewal* |
| 2. Test duration: | 10 d |
| 3. Temperature: | 20 or 25°C |
| 4. Salinity: | 0 ‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m²/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size: | 300 mL minimum |
| 9. Test solution volume: | 100 mL sediment minimum; overlying water variable depending on test type |
| 10. Sediment depth: | 2 cm minimum |
| 11. Renewal of test solutions: | None* |
| 12. Age of test organisms: | 1st - 2nd Instar |
| 13. No. organisms per test chamber: | 10 minimum |
| 14. No. replicate chambers per concentration: | 5 minimum |
| 15. No. organisms per concentration: | 50 minimum |
| 16. Feeding regime: | Variable (None, Tetramin, YCT*) |
| 17. Test chamber cleaning: | None |
| 18. Test solution aeration: | Trickle-flow (< 100 bubbles/min.) |
| 19. Dilution water: | Variable |
| 20. Test concentrations: | Site sediment, a reference sediment and a control sediment |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Survival |
| 23. Sampling and sample holding requirements: | <6 wk |
| 24. Sample volume required: | 4 L |
| 25. Test acceptability criterion: | ≥ 70% survival in controls |

* Slurry of Yeast, Cereal flakes, Trout chow.

**REFERENCES:**

Ingersoll, C.G. and M.K. Nelson. 1990. Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus riparius* (Diptera). pp. 93-109. In W.G. Landis and W.H. van der Schalie, eds., Aquatic Toxicology and Risk Assessment: Thirteenth Volume. ASTM STP 109b. American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1991. New standard guide for conducting solid-phase sediment toxicity tests with freshwater invertebrates. ASTM Draft Document E1383. American Society for Testing and Materials, Philadelphia, PA.

- **Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (DO). For static renewal tests the overlying dilution water should be changed every 48 h at a minimum.**

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE POLYCHAETE, *Nereis virens*, SEDIMENT BIOACCUMULATION TESTS

| | |
|---|---|
| 1. Test type: | Flow-through or Static Renewal |
| 2. Test duration: | 28 d |
| 3. Temperature: | 10 to 20°C |
| 4. Salinity: | ≥ 20‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m$^2$/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D, 14L/10D, 12L/12D |
| 8. Test chamber size: | 1 L (beaker) or large chamber with multiple worms composited into a single replicate (e.g., 20 worms in 20 gallon aquarium) |
| 9. Test solution volume: | > 750 mL/worm |
| 10. Sediment depth: | ≥ 4 cm |
| 11. Renewal of test solutions: | Flow-through = 5-10 vol/d; Static Renewal = 3x/week |
| 12. Age of test organisms: | adult (3 - 15g) |
| 13. No. organisms per test chamber: | One per 1 L beaker, 20 per 20 gallon aquarium |
| 14. No. replicate chambers per sediment: | 5-8 (depending on desired statistical power) |
| 15. No. organisms per sediment: | 5-8 (assumes values to be determined on individuals) |
| 16. Feeding regime: | None |
| 17. Test chamber cleaning: | As needed |
| 18. Test solution aeration: | Moderate, as needed |
| 19. Dilution water: | Natural seawater or modified GP, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water |
| 20. Test concentrations: | Site sediment, a reference sediment and control sediment |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Bioaccumulation |
| 23. Sampling and sample holding requirements: | <8 wk |
| 24. Sample volume required: | 200 mL per worm |
| 25. Test acceptability criterion: | Adequate mass of organisms at test completion for detection of target analyte(s) |

**REFERENCE:**

Lee II, H., B. Boese, J. Pelletier, M. Winsor, D. Specht and R. Randall. 1989. Guidance Manual: Bedded Sediment Bioaccumulation Tests. EPA/600/x-89/302. U.S. Environmental Protection Agency. 232 pp.

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE OLIGOCHAETE, *Lumbriculus variegatus*, SEDIMENT BIOACCUMULATION TESTS

| | |
|---|---|
| 1. Test type: | Static Non-renewal* or Overlying Water Renewal |
| 2. Test duration: | 28 d |
| 3. Temperature: | 20 - 25°C |
| 4. Salinity: | 0 ‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/m$^2$/s (50-100 ft-c) |
| 7. Photoperiod: | 16L/8D |
| 8. Test chamber size: | 4 L minimum |
| 9. Test solution volume: | 1 L |
| 10. Sediment depth: | 3 cm |
| 11 Renewal of test solutions: | Variable |
| 12. Age of test organisms: | Mixed Age Adults |
| 13. No. organisms per test chamber: | 5 g (~500-1000) (Minimum) |
| 14. No. replicate chambers per sediment: | 4 minimum |
| 15. No. organisms per sediment: | N/A |
| 16. Feeding regime: | None |
| 17. Test chamber cleaning: | None |
| 18. Test solution aeration: | If needed to maintain DO> 40% saturation (< 100 bubbles/min.) |
| 19. Dilution water: | Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent, deionized water and reagent grade chemicals or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness |
| 20. Test concentrations: | Site sediment, a reference sediment and a control sediment |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Bioaccumulation |
| 23. Sampling and sample holding requirements: | <6 wk |
| 24. Sample volume required: | 4 L |
| 25. Test acceptability criterion: | Adequate mass of organisms at test completion for detection of target analyte(s) |

**REFERENCES:**

Ankley, G.T., R.A. Hoke, D.A. Benoit, E.N. Leonard, C.W. West, G.L. Phipps, V.R. Mattson and L.A. Anderson. 1993. Development and evaluation of test methods for benthic invertebrates and sediments: effects of flow rate and feeding on water quality and exposure conditions. Arch. Environ. Contam. Toxicol. 25:12-19.

Phipps, G.L., G.T. Ankley, D.A. Benoit and V.R. Mattson. 1993. Use of the aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. Environ. Toxicol. Chem. 12:269-279.

* Static renewal, intermittent flow or continuous flow tests may be used where it is necessary to maintain water quality parameters, e.g., dissolved oxygen (D.O.) and where ammonia is a water quality parameter of concern (cf. Section 11.2.2).

## SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE MACOMA CLAM, *Macoma nasuta*, SEDIMENT BIOACCUMULATION TESTS

| | |
|---|---|
| 1. Test type: | Flow-through or Static Renewal |
| 2. Test duration: | 28 d |
| 3. Temperature: | 12 - 16°C |
| 4. Salinity: | ≥ 25‰ |
| 5. Light quality: | Ambient Laboratory |
| 6. Light intensity: | 10-20 uE/$m^2$/s (50-100 ft-c) |
| 7. Photoperiod: | 12L/12D, 16L/8D, 10L/14D |
| 8. Test chamber size: | 250mL - 1 L (beaker) |
| 9. Test solution volume: | > 750 mL/beaker (e.g., ten 250 mL beakers in 8L aquarium) |
| 10. Sediment depth: | ≥ 50 g wet wt sediment per g wet flesh (without shell) |
| 11. Renewal of test solutions: | Flow-through = 5-10 vol/d; Static Renewal = 3 x/wk |
| 12. Age of test organisms: | 2 - 4 yr, 28 - 45 mm shell length |
| 13. No. organisms per test chamber: | One (1) per beaker maximum |
| 14. No. replicate chambers per sediment.: | 5 - 8 (depending on desired statistical power) |
| 15. No. organisms per sediment: | 5 - 8 (assumes values to be determined on individuals) |
| 16. Feeding regime: | None |
| 17. Test chamber cleaning: | As needed |
| 18. Test solution aeration: | Moderate, as needed |
| 19. Dilution water: | Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water |
| 20. Test concentrations: | Site sediment, a reference sediment and a control sediment |
| 21. Dilution series: | N/A |
| 22. Endpoint: | Bioaccumulation |
| 23. Sampling and sample holding requirements: | <8 wk |
| 24. Sample volume required: | 8 L |
| 25. Test acceptability criterion: | Adequate mass of organisms at test completion for detection of target analyte(s) |

**REFERENCES:**

Lee II, H., B. Boese, J. Pelletier, M. Winsor, D. Specht, and R. Randall. 1989. Guidance Manual: Bedded Sediment Bioaccumulation Tests. EPA/600/x-89/302. 232 pp.

Ferraro, S., H. Lee II, R. Ozretich, and D. Specht. 1990. Predicting bioaccumulation potential: A test of a fugacity-based model. Arch. Environ. Contamin. Toxicol. 19:386-394.

9/5/02

# APPENDIX VII.

# PORE WATER COLLECTION PROCEDURE

# APPENDIX VII

## Collection of Porewater:

Total and unionized ammonia must be analyzed on the sediment interstitial water using the ion-selective electrode method (Merks 1975) following the manufacturer's instructions or the colorimetric method as described in (Bower and Holm-Hansen (1980). Interstitial water should be extracted by centrifuge using the method described in Burgess et al. (1993). Here, 200 ml of sediment are placed in a 250 ml Teflon centrifuge tube and centrifuged at 4°C for 3 h at 4,000 rpm (2520 G). Burgess (personal communication) indicated that, in most cases, 1 h may be adequate. In general, about 20 ml of interstitial water would be needed.

## Analysis of Ammonia:

Total Ammonia may be analyzed using the ammonia probe method (Merks 1975), or the colorimetric method (Bower & Holm-Hansen 1980). Acceptable detection limits are 0.1 mg/L. Unionized Ammonia can be calculated using the dissociation model of Whitfield (1972) as programmed by Hampson (1977). All samples require triplicate analysis.

9/5/02

# APPENDIX VIII

## PROCEDURES FOR ADDRESSING AMMONIA PRESENCE IN *MYSIDOPSIS* SEDIMENT TOXICITY TESTS (ELIZABETH SOUTHERLAND MEMO TO MARIO P. DEL VICARIO, DATED JUNE 14, 1994)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 14 1994

MEMORANDUM

OFFICE OF WATER

SUBJECT: Recommendations for Conducting Sediment Toxicity Test with Mysidopsis bahia when Ammonia may be Present at Toxic Levels

FROM: for Elizabeth Southerland, Acting Director
Standards and Applied Science Division (4305)
Office of Science and Technology

TO: Mario P. Del Vicario, Chief
Marine and Wetlands Protection Branch
U.S. EPA Region 2

The purpose of this memorandum is to provide guidance to U.S. EPA Region 2 on conducting the mysid ten-day solid phase sediment toxicity test to evaluate dredged material for open water disposal. This guidance is provided in response to a letter mailed to Region 2 on April 22, 1994 from Monte Greges, U.S. Army Corps of Engineers, New York District, requesting guidance on running the mysid test when ammonia is present at potentially toxic concentrations.

The Office of Science and Technology held a conference call on May 16, 1994 with EPA and U.S. Army Corps of Engineers scientists and our consultants to develop an acceptable protocol for running the mysid test when ammonia may be present at toxic levels. The following protocol was recommended by conference call participants who are identified below as recipients of this memorandum.

1. The Corps of Engineers and EPA issued joint guidance on December 21, 1993 offering recommendations, based on the best available information, for reducing ammonia levels in test systems used for acute amphipod sediment bioassays. When running mysid tests, it is recommended that the procedure described in the December 21 memorandum be used with modifications pertaining specifically to Mysidopsis bahia.

2. The Corps of Engineers/EPA December 21 guidance memorandum states that at certain open-water dredged material disposal sites (e.g. dispersive situations and situations with well-oxygenated overlying water), ammonia and hydrogen sulfide

may not be contaminants of concern. If chemical evidence of ammonia is present at toxicologically important levels (i.e. ammonia concentrations exceeding the species-specific acceptability ranges), and ammonia is not a contaminant of concern, the laboratory analyst running the mysid ten-day sediment toxicity test should reduce ammonia in the in the test system overlying water to the appropriate acceptable level before adding the test organism.

3. For Mysidopsis bahia, the species-specific acceptable level for unionized ammonia concentration in the test system overlying water (i.e sublethal water column concentration for a ten-day sediment test) is 0.6 mg/L in tests run at 26±1°C, 31± g/Kg salinity, and pH of 7.9-8.0 using one day old organisms . At a test pH of 7.5, the acceptable concentration of unionized ammonia is 0.3 mg/L. These acceptability levels were derived on the basis of acute toxicity tests conducted with ammonia by D.C. Miller, S. Poucher, J.A. Cardin, and D. Hansen at EPA's Environmental Research Laboratory, Narragansett, Rhode Island.

4. If unionized ammonia levels in the test system overlying water exceed the acceptability level for Mysidopsis bahia (0.6 mg/L at pH 7.9-8.0 or 0.3 mg/L at pH 7.5) the system should be flushed at a rate of two volume replacements per day until it reaches a concentration of unionized ammonia at or below the acceptability level. Overlying water should be aerated during flushing, and the analyst should measure the overlying water ammonia concentration each day until the acceptable concentration is reached. Overlying water should be sampled approximately 1 cm above the sediment surface.

5. After adding the test organisms to the system, 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. It is recommended that overlying water concentration of ammonia be measured again at the end of the test.

6. Accurate measurement of sample pH is crucial in the calculation of the unionized ammonia fraction. EPA's Narragansett laboratory recommends the use of specific equipment and procedures for determining pH of seawater (see Attachment 1)

We are sending this memorandum concurrently to EPA Region 2 and the conference call participants who recommended guidance. We ask that conference call participants provide any comments or modifications of the recommended procedure to Tom Armitage of my staff by June 24, 1994. We will notify Region 2 if any changes in the guidance are required.

Attachment

cc: Bob Engler (COE WES)
Tom Dillon (COE WES)
David Moore (COE WES)
Monte Greges (COE NY District)
Gary Ankley (EPA ORD)
Don Miller (EPA ORD)
Norm Rubenstein (EPA ORD)
Rick Swartz (EPA ORD
Tom Chase (EPA OWOW)
Alex Lechich (EPA Region 2)
Joel O'Conner (EPA Region 2)
Dave Tomey (EPA Region 1)
John Scott (SAIC)

ATTACHMENT 1

Use of criteria for developing water quality-based permit limits and for designing waste treatment facilities requires the selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of these criteria (U.S. EPA 1985b). Limited data or other considerations might make their use impractical, in which case one should rely on a steady-state model (U.S. EPA 1986).

## IMPLEMENTATION

Water quality standards for ammonia developed from these criteria should specify use of environmental monitoring methods which are comparable to the analytical methods employed to generate the toxicity data base. Total ammonia may be measured using an automated idophenol blue method, such as described by Technicon Industrial Systems (1973) or U.S. EPA (1979) method 350.1. Un-ionized ammonia concentrations should be calculated using the dissociation model of Whitfield (1974) as programmed by Hampson (1977). This program was used to calculate most of the un-ionized values for saltwater organisms listed in Table 1 and 2 of this document. Accurate measurement of sample pH is crucial in the calculation of the un-ionized ammonia fraction. The following equipment and procedures were used by EPA in the ammonia toxicity studies to enhance the precision of pH measurements in salt water. The pH meter reported two decimal places. A Ross electrode with ceramic junction was used due to its rapid response time; an automatic temperature compensation probe provided temperature correction. Note that the responsiveness of a new electrode may be enhanced by holding it in sea water for several days prior to use. Two National Bureau of Standards buffer solutions for calibration preferred for their stability were (1) potassium

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May 20, 1994

Subject: Mysid No Effect $NH_3$ Concentration for Lethality and pH Issues for Sediment Toxicity Test Protocols
From: Don C. Miller
Research Aquatic Biologist, ERL,N
To: Tom Armitage
Office of Science and Technology (4305)

The following information is provided in response to the May 16, 1994 conference call on sediment toxicity testing where high concentrations of ammonia are present. No mysid tests are directly applicable to estimate a 10 day no lethal effect concentration for $NH_3$. However, data for other exposure periods are available.

1. We believe that 0.6 mg $NH_3$ /L in the water column should be sublethal for 10 day sediment tests with one day old *Mysidopsis bahia* at 26±1° C, 31±1 g/Kg salinity and a pH of 7.9-8.0. At a test pH of 7.5, the sublethal concentration should be approximately 0.3 mg $NH_3$/L.

The 0.6 mg/L value is supported by:

a. four day acute results for Test 16, per J. Cardin 8/15/86 memo, attached. Test 16 pertains to the present question as it was conducted at the above conditions. The LC50 is 1.7 mg $NH_3$/L. The 7% mortality observed in the 0.95 mg/L treatment probably is not significant and may be a no effect concentration for a four day test. For 10 day sediment tests, the lower treatment concentration (0.58 mg/L) may be required because the 10 day continued exposure may result in mortality at lower concentrations.

b. a 32 day chronic value, 0.232 mg $NH_3$ /L, which represents a lower bound no effect concentration (Miller et al., 1990, attached). This value is based on a significant effect on survival at 0.331 mg/L at the same test conditions as above This lower protection concentration reflects the greater sensitivity of mysids after maturation and young begin to develop in the brood pouch. Since eggs do not appear until day 12 to 14 (at 25°), the lower chronic value should not be applied to 10 day sediment tests, assuming one day old animals are used.

The recommended 0.3 mg $NH_3$ /L at pH 7.5 is supported by acute tests at pH 8.0 and 7.0 (Figure 2B, Miller, et al.). These results suggest mysid acute sensitivity to ammonia may increase as much as two-fold at pH 7.5, relative to pH 8.0, hence requiring the 50% reduction in the concentration expected to be sublethal.

2. Also important, but not specifically stated in the subject protocol, are the precautions

necessary to accurately measure pH in seawater. Accurate calculation of $NH_3$ concentrations in the test water requires accurate pH measurement. However, measuring pH in sea water is not straight forward, as indicated in Miller et al. (See discussion, first paragraph). Enclosed is a recommended procedure from the implementation section of the EPA saltwater criteria for ammonia. We suggest this issue be highlighted in the protocol.

3. Should additional studies be desired to better describe the $NH_3$ no effect concentration for mysids, we recommend: (a) flow through testing, using a pH controller, or at a minimum, 24 h monitoring of pH during day one, and (b) the tests be conducted for the range of pH conditions expected in sediment testing. The variance shown in the attached paper (Figure 2B) for static tests is due to pH drift in tests which were not monitored over night. In contrast, Figure 2A shows good agreement may be achieved with flow through tests where there was 24 h monitoring of pH during day one.

attachments: Cardin memo
Miller et al. paper
$NH_3$ criteria implementation

cc without attachments: N. Jaworski
G. Pesch

9/5/02

# APPENDIX IX.

# AED LABORATORY OPERATING PROCEDURE, MEASUREMENT OF TOTAL LIPIDS USING MODIFIED BLIGH-DYER METHOD

# AED LABORATORY OPERATING PROCEDURE MEASUREMENT OF TOTAL LIPIDS USING MODIFIED BLIGH-DYER METHOD.



**POINT OF CONTACT:**

Environmental Chemistry Group
Atlantic Ecology Division
U.S. Environmental Protection Agency
27 Tarzwell Drive
Narragansett, RI 02882

## 1. OBJECTIVE

This document defines a procedure based on a modification of the method reported by Bligh and Dyer (1959). This procedure is used to analyze marine tissues for total lipid content.

## 2. MATERIALS

Solvents

Methanol - Baxter Pesticide Grade
Chloroform - Baxter Pesticide Grade (ethanol free)
Deionized water

Glassware

TurboVap tubes, 25ml scintillation vials, and 50ml centrifuge tubes muffled at 450 degrees F for 6 hours.

Equipment

Mayer N-Evap Analytical Evaporator
Zymark TurboVap Evaporator
Sorvall RC2-V Centrifuge
Kinematica Homogenizer with 12mm tip.

## 3. ANALYTICAL PROCEDURE

All Trophic Transfer samples were stored at -20 degrees c immediately after collection and thawed just prior to analysis. Solvent ratios in the following procedure are expressed in the order: **chloroform/methanol/water**.

3.1) For lobster muscle, place 10g wet homogenized tissue in a 50ml centrifuge tube. For lobster hepatopancreas and Nereis tissue place 5g wet homogenized tissue in a tared 25ml scintillation vial.

3.2) Calculate the amount of water that is in the sample by using the formula:[ grams wet x (1 - dry/wet ratio)] = (ml)water. The (ml)water is used to calculate the appropriate amounts of chloroform and methanol to add to the centrifuge tube to obtain a **solvent volume ratio of 1/2/0.8**. Thus, to calculate the amount of chloroform needed for 4ml of water in the sample, multiply 4ml x 1.25 = 5ml chloroform and 2 x chloroform = 10ml methanol. The ratio of chloroform/methanol/water in the centrifuge tube or vial is now 5/10/4 or **1/2/0.8** . Add the appropriate amounts of chloroform and methanol to the centrifuge tube and blend with a 12mm polytron tip for 60 seconds.

3.3) Add an additional volume of chloroform to the centrifuge tube/vial that is equal to the amount used in step 2. Blend for 30 seconds.**(Solvent volume ratio 1/1/0.4)**

3.4) Add an additional volume of water to the centrifuge tube/vial that is equal to the amount calculated in step 2. Blend for 30 seconds. **(Solvent volume ratio 1/1/0.9)**

3.5) Cap the tube/vial and centrifuge for 10 minutes. Draw off the chloroform and dispense it into a turbovap tube for muscle tissue or a 25ml scintillation vial for hepatopancreas and Nereis tissue.

3.6) Rinse all transfer tools with small portions of chloroform, collecting the washes in the centrifuge tube or scintillation vial.

3.7) Add an additional volume of chloroform equal to 2 times the amount used in step 2 to the remaining tissue in the centrifuge tube or vial. Blend for 30 seconds. **(Solvent volume ratio 1/1/0.9)**

3.8) Cap the tube/vial and centrifuge for 10 minutes. Draw off the chloroform and transfer to the turbovap tube. Rinse transfer tools with small portions of chloroform into the tube or vial.

3.9) Repeat steps 7 and 8 except shake manually instead of using the Polytron.

3.10) If the extract is cloudy or contains an emulsion, pass it through a layer of sodium sulfate and collect. Repeat as needed to clarify extract. Rinse apparatus with small portions of chloroform.

3.11) For muscle tissue, volume reduce the extract under a nitrogen stream in the turbovap tube to 1ml then transfer to a 25ml scintillation vial and blow to dryness under nitrogen in an N-Evap evaporator. For hepatopancreas and Nereis tissue extracts (which are already in a 25ml scintillation vial) reduce to dryness in the N-Evap evaporator.

3.12) Place the uncapped scintillation vial in an oven at 100 degrees c for 1 hour then allow the vial to cool in a desiccator for 15min and weigh.

3.13) Calculate the weight percent of total lipid in the sample using the formula: ((g)lipid / (g)dry sample weight) * 100 = percent lipid.

## 4. REFERENCES

Bligh, E.G. and W.J. Dyer. 1959. Canadian Journal of Biochemistry and Physiology, 37(8), pp. 2-8.