2.0 LABORATORY METHODS, QUALITY ASSURANCE, AND QUALITY CONTROL FOR SEDIMENT QUALITY ANALYSIS - TOXICITY TESTS

Biological sediment characterizations (bioassays) will be conducted to test and evaluate the sediment samples relative to Washington State Sediment Management Standards (WAC 173-204) . The following sediment bioassays will be conducted on four test samples obtained from Middle Waterway at a depth of 0- 10 cm, and appropriate reference and control samples as described in WAC 173-204 or associated guidance.

- 10-day amphipod bedded sediment test using *Rhepoxynius abronius* or *Ampelisca abdita*
- 20-day polychaete growth test using *Neanthes arenaceodentata*
- The echinoderm larval sediment elutriate test using *Dendraster excentricus*
- The Microtox® Saline-extract test.[1]

Procedures for testing, analysis, quality assurance and quality control are discussed below. Procedueres for sampling and handling of sediments are included in the Sampling and Analysis Plan; in general, stations shall be accessed from shore via foot. Grab samples will be obtained within 1 meter of the sampling station for bioassays by using stainless steel spoons and bowls and sample material will be transfered to 1 liter sampling jars for transportion to the testing laboratory.

2.1 Quality Assurance Procedures

To ensure the production of technically defensible biological data, a QA/QC program will be instituted as part of the biological characterization of test sediments. This program has included a competitive laboratory selection process for the contracting laboratory, and the utilization of quality assurance and quality control protocols developed for biological analysis by the contracting lab. The elements of this QA/QC program are discussed below.

2.1.1 Selection of Bioassay Laboratory

Parametrix, Inc. of Kirkland, Washington was selected to perform bioassays following the competitive selection process. Parametrix has an extensive record of performing bioassay tests required for biological assessment, dredge disposal and pre-remedial design.

2.1.2 Quality Assurance Program Plan and Test Protocols

The QAPP prepared by Parametrix and Northwest Aquatics (Newport, OR) for the analysis of test sediments in the Hylebos waterway are being utilized as the Quality Assurance Project Plans for this project, with appropriate modifications for project scale. This QAPP has not been reproduced here, but is instead summarized with deviations noted. Parametrix has a copy of the QAPP at their Kirkland facility.

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[1]Criteria established under PSDDA will be utilized for Microtox testing.

2.1.3 Quality Control

Quality control checklists will be used by the laboratory to ensure that all procedural and data elements of the tests will be followed and recorded. An example of these checklists is included here as an attachment; the checklists also include specific bench data sheets. These checklists have been recommended for use by the U.S. Army Corps of Engineers in the *QA/QC Guidance for Laboratory Dredged Material Bioassays* (USACOE WES 1993, Draft).

For each batch of bioassays, the lab will initiate these checklists. Lab staff are required to complete all elements of the checklists, and the original lists will be submitted as a deliverable in the final data package.

2.2 Test Procedures

General guidance for conducting biological testing in Puget Sound may be found in the revised Puget Sound Estuary Protocols (PSEP 1991), with applicable modifications identified under the PSDDA (1990) program. The following sections discuss both general and test-specific methods and performance criteria.

2.2.1 General

All general criteria defined by PSEP (1991) will be applied to this program. In addition, the following project-specific criteria will be used:

- All tests will be conducted within 8 weeks from the time of sediment collection. Holding conditions will be 4°C in the dark. Samples with any remaining head space will be stored under nitrogen.
- All physical/chemical measurements will be taken from a surrogate sixth replicate at the time of inoculation, and at the conclusion of the amphipod, *Neanthes,* and *D. excentricus* tests.
- The lab will incorporate a completely randomized design for replicate placement in water baths or growth chambers.
- Total ammonia and sulfides will be measured at the time of inoculation and at test termination for the amphipod, *Neanthes*, and *D. excentricus* tests.
- Positive control tests that exceed the UWL or UCL will be brought to the immediate attention of the City of Tacoma project coordinator.

2.2.2 Control and Reference Sediments

Control sediments for most bioassay testing will be collected from West Beach (Whidbey Island, WA). Control sediments for *Ampelisca abdita* will come from the test organisms' collection site. Control tests are used to assess the relative health of the test species. During late summer and early fall, West Beach control sediments may experience unusual test mortality. To reduce the chance of test failure, the West Beach control sediments may be gently washed to remove organic material. In past years, use of this procedure for the PSDDA program has reduced control mortality to levels typical of the rest of the year. In the event that test sediments are washed, a second set of unwashed control sediments will also be tested.

Reference sediments for bioassay testing will be collected from within Carr Inlet, based upon the recommendation of the Corps DMMO. Reference sediments will contain approximately the same sediment grain size (i.e., percent fines) as the test sediment. To ensure a reasonable grain size match, potential reference sediments will be wet sieved during collection. Results of wet-sieving that are within the range of percent fines ±10 percent will be considered acceptable. Reference sediments will be analyzed for grain size, total organize carbon, total sulfides, total solids, total volatile solids, and ammonia using methods provided in the Sampling and Analysis Plan. Additional sediment will be archived for potential chemical analysis. This sediment could be analyzed if unexplainable reference sediment failures were noted.

Performance criteria for control and reference sediments are provided in Tables 2-1 thorugh 2-4. If these criteria are exceeded, the contracting laboratory will notify the City project coordinator, who will in turn notify the EPA project coordinator in order to evaluate of the data. In past PSDDA projects, there have been occasions when control sediments have slightly exceeded the criteria but reference and test sediments have both passed. Based on best professional judgment, the PSDDA agencies accepted the data. In the event that similar situations arise during this analysis, best professional judgment will be applied, in consultation with EPA, the natural resources agencies and, as necessary, the DMMO, to determine whether the test results pass the corresponding criteria.

2.2.3 Ten-Day Amphipod Bedded Sediment Test

These tests will be conducted with either *Rheopoxynius abronius* or Ampelisca abdita, depending upon the physical conditions of the test sediments. *R. arbronius* is the preferred test species and will be used on all test sediments having a combined percent fines (silts + clays) of $\leq$ 60 percent. *A abdita* will be used for those sediments having percent fines > 60 percent. The decision criteria for determining test performance (i.e. pass/fail) will be applied uniformly to both species.

A summary of the test conditions and test acceptability criteria for the amphipod test are found in Table 2-1. Taxonomic verification of the test organisms will be conducted on specimens from at least one collection or shipment.

2.2.4 Echinoderm Larval Test

These bioassays will be conducted using larvae of the eastern Pacific sand dollar, *Dendraster excentricus*. Test conditions and acceptability criteria for this procedure are found in Table 2-2. Program-specific procedures and criteria for the *D. excentricus* are as follows:

- All seawater used in the larval test must be collected within 48 hours of use in the tests.
- For each control, reference, and test replicate, three 10-milliliter aliquots will be withdrawn and preserved at test termination. Two of those aliquots will be counted and the data submitted with the final report. The third aliquot will be archived by the City for a period of up to one year beyond the submittal of the final data package.

2.2.5 20-Day *Neanthes* Growth Test

N. arenaceodentata is the test organism for this bioassay. Test conditions and acceptability criteria for this procedure are found in Table 2-3. Particular attention will be given to ensuring that the specified initial age and weight of the test organisms are observed. There are no additional special conditions attached to this test.

2.2.6 Microtox Saline-Extract Test

Test conditions and acceptability criteria are found in Table 2-4. In conducting this analysis, a dilution series is run on the sediment extract, and a total of five replicates are required at the highest dilution concentration. Reference material is to be run with each batch, with a batch being defined as all tests conducted on a single lyophilized vial of test bacterium. Tests will be conducted within 6 hours of reconstituting the bacteria.

2.3 Data Reporting Requirements

Upon completion of all testing, the lab will submit a report that includes the data listed below. The report will be provided both in hard copy and magnetic media (DOS-compatible).

- Survival of test organisms in each test container expressed as the number of test organisms alive, number dead, number missing, and the proportion surviving.
- The mean percent survival, standard deviation, and variance for each test sediment.
- For the echinoderm test, number of normal and abnormal larvae recovered from each test vessel.
- For the *Neanthes* growth test, raw data including average weight of test organisms recovered in each test vessel.
- For the Microtox$^{\otimes}$ test, raw data including average weight of test organisms recovered in each test vessel.

- Water quality measurements, including ammonia and sulfides. Accompanying the ammonia and sulfide data, the lab will also supply the associated instrument calibration and results for seawater spikes.
- Interstitial water salinity values.
- 96-hour LC_{50} values with 95 percent confidence intervals for the reference toxicant. Method of calculating the LC_{50} will also be included.
- Results of any priority pollutant scan(s) conducted on the seawater used in the tests.
- Any problems or deviations from the protocols, SOPs, or the SAP that may influence test results or data quality.
- Copies of all lab QC checklists for bioassay.

2.4 Quality Assurance Review of Lab Data

All data developed by the laboratory will be subject to a quality assurance review. QA guidelines for bioassay data review procedures that will be followed in this program are adapted from Sturgis (199), PTI (1989),and WEST (draft, 1993). An example of the QA review checklist is included here as an attachment. At a minimum, the submitted data will be reviewed for the following.

- **Data Completeness**. Defined as the amount of data obtained versus the amount of data originally intended to be collected. For this program, 80 percent will be considered acceptable.
- **Data Quality Objectives**. Data will be reviewed for compliance with the acceptable parameters established in the specific test protocols. These may include, but are not limited to the following:
    - Test conducted within specified holding times
    - Test organism normalities/abnormalities exceeding performance criteria
    - Out-of-range water quality parameters
    - Lack of randomization
    - Lack of required reference, control, or reference toxicant exposures
    - Reference toxicant results outside of specified ranges.

2.5 Corrective Action for Unacceptable Data

Tests that do not meet completeness and DQO objectives will either be qualified or be rerun. The conditions under which data will be qualified or tests rerun are shown in Table 2-5.

Table 2-1. Summary of test conditions and test acceptability criteria for *Rhepoxynius abronius* and *Ampelisca abdita*.

Parameter	Description
1. Test Protocol	*R. abronius* PSEP, 1991 with PSDDA (1990) modifications *A. abdita* ASTM Method E1367, adapted to PSDDA modifications
2. Test Duration	10 days
3. Temperature	*R. abronius* 15° ± 1°C *A. abdita* 20° ± 1°C
4. Lighting	Continuous Ambient Lighting (50-80 foot-candles)
5. Test Chamber Size	1 L
6. Volume of Test Sediment	175 mL/replicate
7. Number of Replicates/Test	5
8. Number of Organisms/ Replicate	20
9. Aeration	≤ 100 bubbles/minute
10. Test Water	28 ppt ± 1 ppt
11. Dissolved Oxygen	≥ 5.0 mg/L
12. pH	>7, ≤ 9
13. Daily Observation	Temperature, salinity, dissolved oxygen, pH, and daily emergence. At time 0, every 24 hours thereafter, and test termination. Ammonia and sulfide at time 0, and test termination.
14. Reference Toxicant	Cadmium chloride
15. Endpoints	Recovered animals and reburial at test termination LC50 Reference Toxicant
16. Test Acceptability	Control Mortality ≤ 10% Reference ≤ 25%.

Table 2-2. Summary of test conditions and test acceptability criteria for *Dendraster excentricus*.

Parameter	Description
1. Test Protocol	PSEP, 1991, with appropriate PSDDA (1990) modifications
2. Test Duration	4Variable. Test continues until development to the pluteus stage is achieved in 95% of the individuals in the sacrificial seawater control.
3. Physical Parameters	
Temperature	15 ± 1°C
Salinity	28 ± 1 ppt
Dissolved Oxygen	≥ 7.0, ≤ 9.0
pH	
4. Lighting	14 hr light, 10 hr dark using ambient light
5. Test Sediment Volume	20 gms/1 L seawater
6. Number of Replicates/Test	5
7. Test Water	Test water must be used within 8 hours of collection
8. Number of Organisms/ Replicate	20-30 embryos/mL
9. Settling Time	For *Dendraster*, 4 hr before inoculation, with gentle aeration
10. Water Quality Measurements	Temperature, salinity, pH, DO at times 0, 24 and 48 Ammonia and sulfide at least initiation and termination
11. Reference Toxicant	Cadmium chloride
12. Endpoints	Number of normal and abnormal larvae in Test Replicates. LC50 and EC50 on Reference Toxicant
13. Test Acceptability	Seawater Control Combined Mortality and Abnormality ≤ 50%

Table 2-3. Summary of test conditions and test acceptability criteria for *Neanthes arenaceodentata*.

Parameter	Description
1. Test Protocol	PSEP, 1991 with PSDDA modifications
2. Test Duration	20 days
3. Physical Parameters	
Temperature	20°C ± 1°C
Salinity	28 ± 2 ppt
Dissolved Oxygen	≥ > 7.0, ≤ 9.0
Aeration	150-300 ml/min
4. Lighting	Continuous Ambient Lighting (50-80 foot-candles)
5. Test Sediment Volume	175 mL/1 L/replicate
6. Number of Replicates/Test	5
7. Number of Organisms/ Replicate	5
8. Age of Test Organisms	2-3 weeks post-embryo, 0.5-1.0 mg dry weight
9. Measurement	Dry weights of 3 sets of 5 worms each Ammonia and sulfides from overlying water Salinity, temperature, dissolved oxygen, pH, interstitial salinity adjusted to 28 ppt
10. Test Water Renewal	Every third day, 1/3 volume of each replicate
11. Feeding Regime	Dried, powdered *Ulva* or *Enteromorpha*, or Tetramarin⊗ 8 mg/juvenile every other day
12. Water Quality Measurements	Test initiation, prior to renewal event (except ammonia and sulfides), and test termination
13. Reference Toxicant	Cadmium chloride
14. Endpoints	Mortality, final dry weights LC50 on Reference Toxicant
15. Test Acceptability	Control Mortality ≤ 10% Reference Biomass ≥ 80% of the Control Biomass

Table 2-4. Summary of test conditions and test acceptability criteria for saline-extract Microtox procedure.

Parameter	Description
1. Test Protocol	PSEP, 1991 with PSDDA modifications
2. Test Duration	15 minutes
3. Replication	5 at the highest concentration 2 at each subsequent dilutions
4. Reference Toxicant	Phenol or ethanol
5. Frequency of Reference Sediments	One per lot of bacterial (all vials shipped together) or one per every 20 samples, whichever is less
6. Centrifugate holding time	2 hr
7. Endpoints	Light readings and gamma calculations for all replicates. Calculation of EC50 for reference toxicant.
8. Test Acceptability	Confirmation of dose response in reference toxicant and calculated EC50 within 2 standard deviations of the lab's performance chart for Microtox. Reference sediment performance is $\leq$ 20% blank-corrected light reduction

Table 2-5. Summary of test deviations and suggested responses. (Adopted from USACE, 1993-DRAFT)

Deviation	Corrective Measures	
	Retesting Required	Retesting May Be Required[1]
Lack of test array randomization		√
Testing was not blind		√
Required references or controls were not tested	√	
Test chambers not identical		√
Test container(s) broken or misplaced		√
Test organism mortality in controls or reference exceeds acceptable limits	√	
Excessive test organism mortality in a single replicate of a control		√
Test organisms were not randomly assigned to test chambers		√
Test organisms were not from the same population		√
Text organisms were not all the same species (or species complex)	√	
Test organism holding times were exceeded		√
Water quality parameters consistently out of range	√	
Brief episodes of out-of-range water quality parameters		√
Test monitoring was not documented		√
Test monitoring was incomplete		√
Sediment holding times were exceeded	√	
Sediment storage conditions were out of acceptable ranges		√[2]

[1] If not retested, data may have to be qualified

[2] Unless evidence is provided to show that sediment quality) geochemistry and contaminated levels) have not been affected

EXAMPLE QUALITY CONTROL CHECKLIST FOR BIOASSAYS

PROJECT SCHEDULE

Sediment Collection and Expiration Dates

Date of First Sediment Collection _____

Date Sediment Delivered _____

Holding Time (check one) 2 weeks _____

8 weeks _____

Holding Time Expiration _____

Amphipod Collection and Handling Conditions

Date of Amphipod Collection: _____

Site of Amphipod Collection _____

Field Personnel _____

Collection site salinity _____

Collection site temperature _____

Field weather conditions _____

Time initiated - time completed _____

Time Arrive Lab: _____

Water Temperature at return _____

Storage Facility: _____

Storage Temp: _____

Buckets Aerated: _____

Field Notes: _____

Exposure Dates

Test Setup _____

Amphipod Innoculation _____

Test Breakdown _____

Reporting Requirements

Data available for report compilation _____

Draft Report completed _____

QA Review by: _____

Report Due to Client By: _____

mphipod Innoculation

Collect amphipods by sieving a small amount of sand through a 1.0 mm NITEX screen in seawater. Take screen out of water for just a moment. and then place back into the seawater. The amphipods will float. and are available for collection with a glass or plastic beaker.

Sort 10 sexually immature amphipods approximately 4mm into a plastic cup, with 1/4 in. of seawater and keep on ice. DO NOT collect obvious females with brood pouch. or sexually active males.

QC Amphipods with dissecting microscope. DO NOT use Stage Lighting System. _____

QA counts in cups prior to innoculation. _____

Monitor physical parameters (DO. pH, salinity) in all vesels prior to innoculation. _____

Combine Amphipods into groups of 20 placing the empty cup on the bottom of the full cup. Being careful not to leave any in the empty cups. _____

To seed Amphipods first remove all the watch glasses from one row of test vessels. Next seed the vessel farthest away, Check to see that all amphipods sink into water column nd are not retained in the medicine cup. replace the watch glass and place the empty medicine cup on top of it. Proceed to the next test vessel

Seed reference toxicant replicates _____

Allow Amphipods one hour to rebury in test sediment if they do not bury. _____

Remove them using a clean pippet and replace them with a healthy amphipod _____

Check to make sure Ref-Tox Amphipods are not trapped on surface _____

Take Ammonia and Sulfide samples. _____

Each label should contain date. time. organism. Sulfide or Ammonia, test name and number. sample name or number, and initials. _____

Check to make sure watch glasses are placed on ref-tox and amphipods are not trapped on surface _____

Refrigerate Ammonia and Sulfide sample bottles _____

Clean laboratory area _____

Initials of individual verifying completion of tasks _____

AMPHIPOD SETUP FORM

Project
Project Number
Date:

Test Organism
Page _____ of _____

Project Sample I.D. Number	Lab Replicate Number	Interstitial Salinity	Soil Type/Comments	Initials

AMPHIPOD SOLID PHASE BIOASSAY: DAILY OBSERVATION FORM

[illegible] ____________________ Test Start ____________

Project Number ____________________ Test End ____________ Page ______ of ______

Replicate	Amphipods Visible									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10

AMPHIPOD BREAKDOWN PROCEDURAL CHECKLIST

Conduct Final Replicate Physical Monitoring _____

Record Daily Observations _____

Take ammonia and sulfide samples _____
(Each label should contain date, time, organism,
Sulfide or Ammonia, test nameand number, sample name
or number, and initials.)

Store ammonia and sulfide samples at 4 degrees C _____

Screen sediments using .5mm screen _____

Collect amphipods using a pipet and place in labelled medicine cup _____

Make sure Amphipods are kept in an adequate supply of sea water. _____

Place West Beach sand in medicine cups and add Amphipods. placing
.npty Amphipod cup on the bottom. Leave for One Hour to rebury. _____

Record reburial data _____

Confirm all data is correctly entered, no blanks allowed. _____

File all raw data sheets with the project file, and copies with this notebook _____

Remove temperature record sheet and place in notebook _____

Clean laboratory after breakdown. _____

Schedule glass clean-up and decontamination _____

Initials of individual verifying completion of tasks _____

AMPHIPOD BREAKDOWN DATA SHEET

Project ______________ Date ______________________

Project Number ______________ Page ______ of ______________

			Found		
Replicate	Initials	Visible	Alive	Dead	Reburied

AMPHIPOD DAILY WATER QUALITY MONITORING

Project _____
Project Number _____
Page _____ of _____

Test Organism _____
Test Day # _____
Date _____

Instrument Serial Number _____

Replicate	Temperature (degrees-C)	pH	Salinity (o/oo)	D.O. (mg/L)

AMPHIPOD DAILY QC CHECKLIST

Project _____

Project Number _____

Conduct task, and initial after completion

Date:	Day 0	Day 1	Day 2	Day 3
Monitor	____	____	____	____
Emergence	____	____	____	____
Note observations	____	____	____	____
Monitor Ref-Tox	____	____	____	____
Check aeration	____	____	____	____
Restore water levels with D.I. water	____	____	____	____

Date:	Day 4	Day 5	Day 6	Day 7
Monitor	____	____	____	____
Emergence	____	____	____	____
Note observations	____	____	____	____
Monitor Ref-Tox	____			
Check aeration	____	____	____	____
Restore water levels with D.I. water	____	____	____	____
Replace Temp. Record Card				____
Ref Tox Breakdown	____			

Date:	Day 8	Day 9	Day 10
Monitor	____	____	____
Emergence	____	____	____
Note observations	____	____	____
Check aeration	____	____	____
Restore water levels with D.I. water	____	____	____
Remove Temp. Record	____	____	____

Amphipod Reference Toxicant Procedures

or use with *Rhepoxynius abronius & Ampelisca abdita*

Reference toxicant should be prepared as follows:

Positive control: Cadmium Chloride. Express concentrations as Cd.
Stock Solution prepared at 10 mg/L
Stock Preparation Date:_____

Preparation of Reference Toxicant Replicates

Cd Concentation	ml Stock Solution	ml Seawater	Label Replicates
1.5mg/l	0.15	999.85	A - C
75mg/l	0.075	999.925	D - F
.25mg/l	0.025	999.975	G - I
0.0mg/l	0	1000	J - K

reserve 100 mL of Reference Toxicant at highest concentration for analysis _____

Reference Toxicant Replicates Prepared By: _____

AMPHIPOD SETUP PROCEDURAL CHECKLIST

eawater Collection. Filtration. Preparation

Seawater Volume Required	_____
Approximate Volume Collected	_____
Date and Time of Collection	_____
Location of Source Water	_____
Collection Temperature	_____
Filter and Adjust Sea water	_____
Final Seawater Salinity (o/oo)	_____

Randomization Schedule

Randomization prepared by	_____
Place copy of schedule with this file	_____

Sediment Setup

Measure and record interstitial salinity	_____
Sieve control sediment and wash with clean sea water. Use .5mm screen.	_____
Verify temperature of water bath or E.C.	_____
Check to see that the light cycle is set for constant illumination.	_____
Use deconed stainless steel spoons and plasitc cups to dispense 175ml of sediment into each test vessel .	_____
Take ammonia and sulfide for each station	_____
Purge remaining sample containers with Nitrogen	_____
Add adjusted seawater to test vessel to 1000 mL	_____
Aerate all replicates @ < 100 bubbles/minute	_____
Put glass covers on all replicates	_____
Clean up the lab area	_____
Initials of individual verifying completion of tasks	_____

QUALITY CONTROL CHECKLIST FOR 10-DAY AMPHIPOD TEST

SPECIES: (check one)

Rhepoxynius abronius _____
Ampelisca abdita _____

PROJECT DATA

PROJECT NAME: _____

PROJECT NUMBER: _____

CLIENT: _____

CLIENT CONTACT: _____

ADRESS _____

_____

_____

_____

PHONE NUMBER _____

PROTOCOL

Project Testing Program (check one)

PSDDA _____
PSEP _____
Green Book _____
Other _____

Laboratory Protocol Number _____
Protocol Reviewed and Signed by Client? _____

PROJECT STAFF

Principal Investigator _____
Associate Investigator _____
Staff _____

_____

_____

QA Officer _____

_____

Protocol Reviewed by all project staff? _____

DATA QUALITY ASSURANCE CHECKLIST FOR BIOASSAYS

AMHIPOD FINAL QUALITY ASSURANCE CHECK

TEST # EXPER. #

CONTROL % SURVIVAL THIS TEST: PASSED FAILED

Reference Test Experiment #: Control % Surviv.: LC 50:

List any problems associated with this test:

_____

_____

_____

_____

_____

_____

CHECK OF DATA INPUT PAGES

_____ Present: Method Summary sheet Randomization sheet (1-2 pg), Breakdown sheets (1-2 pg), 10 Daily Data sheets. Physical Data sheets (1-2). Field/Culture sheet Holding Time table

_____ Breakdown sheet: verify that vials were recounted.
_____ Breakdown sheet: verify that live animals found during repick were added to the total live pods.
_____ Breakdown sheet: verify that live animals found at 24, 48, 72 hr were added to total of live pods found.
_____ Breakdown sheet: verify that any tubes found at start were included in the total number of animals per rep.
_____ Breakdown sheet: check to see if the sum of the number dead during test and the number found live exceeds 20.
_____ Breakdown sheet: verify that reps in which no animals were found contained no tubes, molts, or animals dead durin
_____ Breakdown sheet: make sure that QA'd sheet was signed.
_____ From Holding Time sheet, verify that experiment numbers, sample numbers, collection dates, and Day 0 dates were
_____ and signed.
_____ From 10 day data sheets, verify the test day numbers on which the physical data were taken.

CHECK OF DATA OUTPUT PAGES

_____ Present: Data Entry Pages (1-2), Summary Data Pages (2), Data Base Pages (1-2) Project Summary Page Stat pages (1-2)

_____ RAND file: From randomization sheet: verify test and experiment number.
_____ RAND file: From randomization sheet: verify jar numbers.
_____ RAND file: From randomization sheet: verify sample numbers.
_____ RAND file: From holding time table: verify days hold.
_____ RAND file: Make sure experiment number appears in footer.

_____ SORT file: Verify that RAND file was QA'd and signed before it was converted to a SORT file.
_____ SORT file: Verifty that the filename has been changed to SORT.
_____ SORT file: From 10 day data sheets: Verify temperature range.
_____ SORT file: From breakdown sheet: verify that correct breakdown sheet was used.
_____ SORT file: From breakdown sheet: verify values for number alive (total live pods at end of test).
_____ SORT file: From breakdown sheet: verify values for the number of pods added to each jar (# per rep).
_____ SORT file: From physical data sheet: verif that corrrect physicl data sheet was used as a source of the pH, D.O., Sali
_____ SORT file: verify that the physical data were entered for the correct two replicate numbers.
_____ SORT file: verify pH values.
_____ SORT file: verify D.O. values.
_____ SORT file: verify salinity values.

CHECKLIST FOR AMPHIPOD MORTALITY BIOASSAY

Project Name: _____ SAIC Project No: _____

Laboratory: _____ Lab Number _____ Batch _____

Responsible Technician _____ Reviewed By: _____

Amphipod species _____

Date Sampled _____ Received by Lab _____

Date Analysis Begun _____

Problems noted (e.g., deviations from prescribed methods, analytical problems)

_____

_____

_____

_____

COMPLETENESS AND HOLDING CONDITIONS

Samples Submitted _____ # Samples Analyzed _____

Holding conditions acceptable (Y/N) PSEP ; 4° C under nitrogen < 2 weeks _____

PSDDA; 4° C under nitrogen < 8 weeks _____

If no, identify samples _____

_____

FORMAT

Standard data report sheet (check off)

Number of amphipods reported for each replicate _____ Field samples _____

Percent Mortality reported for each replicate _____ Positive controls _____

Daily emergence taken for each replicate _____ Negative controls _____

Individual replicate, plus sample mean and standard deviations for mortality? _____

Analytical Replicates

Number per Sample _____

Any < 5 RPD? _____

Water Quality Variable Reported for each Replicate (check)

Interstitial salinity for each sample (initiation) _____ Salinity (daily) _____

Dissolved Oxygen (daily) _____ pH (daily) _____

Temperature (daily) _____ Sulfide (initiation and termination) _____

Ammonia (initiation and termination) _____

CHECKLIST FOR AMPHIPOD MORTALITY BIOASSAY

QA/QC SAMPLES

Negative Control

Control Sediment Collection Site _____
Water Source _____
Current priority pollutant scan available? _____
Mean Control Mortality (%) _____
Exceed PSEP QA Limit of 10%? _____

Reference Sediment

Collection Site _____
Total Number of Analyses _____
Mean Mortality _____
Mean mortality exceed PSEP QA limit of > 20% over control? (Y/N) _____

Positive Controls

Reference Toxicant _____
Exposure Concentrations _____
% mortality/exposure concentration _____
Organism Response (LC50) _____
Laboratory Performance Standards for Reference Toxicant _____
Did the test LC50 fall within lab standards (Y/N)? _____

WATER QUALITY

Samples with temperature <14 or > 16° C _____
Samples with salinity < 27 or > 30 ppt _____
Samples with pH < 7 or > 8 _____
Samples with DO < 5 mg/L _____

AMHIPOD FINAL QUALITY ASSURANCE CHECK

_____ CALC file: Verify that SORT file was QA'd and signed before it was posted into CALC file.

_____ CALC file: verify that reps for which there is no data have an empty cell under decimal mortality.
_____ CALC file: Stats page(s): verify that reps for which there is no data have been replaced by a period.
_____ CALC file: Database pages: Verify that reps for which there is no data have been cleared.
_____ From Breakdown sheet: verify that comments have been transferred to Summary Data Pages.
_____ From 10 Day Data Sheets: verify that comments hvae been transferred to Summary data sheets.
_____ From Physical Data Sheets: verify that comments have been transferred to the Summary Data Sheets.
_____ From the corresponding Reference Test: verify that any comments have been transferred to the

Table 3-1. Benthic laboratory rescreening log.

SURVEY _____

Station and Replicate Number	Area	Date Collected	No. of Bags or Jars	Date Rinsed	No. of Jars per Sample	Initials

Table 3-3. Benthic laboratory QC form for sorting.

SURVEY _____ Page ___ of _____

STATION NAME _____ REPLICATE NO. _____

APPROXIMATE SAMPLE VOLUME _____ SORT QC VOLUME _____

SORTED BY/DATE _____ QC SORTED BY/DATE _____

IDENTIFICATION OF QC BY/DATE _____

INDIVIDUALS FOUND IN THE QC SORT:

Taxon Name	Taxon Code	Count		Comments
		QC	Final	

RESORT INDICATED Yes No By: _____

Table 3-2. Benthic laboratory sorting form.

SURVEY _____

Station	Replicate	No. of Jars	Volume	No. of Hours	Sorted by	Taxonomist's Initials					
						Polychaetes	Arthropods	Mollusks	Ophiuroids	Echinoderms	Miscellaneous

STANDARD OPERATING PROCEDURES
AND
LABORATORY QUALITY ASSURANCE PROJECT PLANS:

BENTHIC INFAUNA ANALYSIS

Sampling, Analysis and QA/QC Procedure Manual

for Field and Laboratory Work-up of Benthic Infauna

for

Marine Taxonomic Services

Field Procedures--

The field and laboratory procedures followed by Marine Taxonomic Services (MTS) are designed to ensure the generation of high quality data. This objective is achieved through careful sample handling, sorting and identification procedures.

Field Procedures

Fixative Preparation--

The fixative most commonly used for benthic macroinvertebrate samples is formalin, an aqueous solution of formaldehyde gas. Under no circumstances is ethyl or isopropyl alcohol (i.e., preservatives) used in place of the formalin. Penetration of the alcohol into body tissues is too slow to prevent decomposition of the specimens.

MTS uses formalin solutions of 5-20 percent (v/v) strength for fixing marine organisms as recommended by Gosner 1971:Birkett and McIntyre 1971; Smith and Carlton 1975; Swartz 1978. Solutions of 10-15 percent are used most commonly.

The formalin solution is always buffered to reduce acidity. Failure to buffer this solution may result in decalcification of molluscs and echinoderms. Ideally, the pH should be at least 8.2, as calcium carbonate dissolves in more acidic solutions. Borax (sodium borate, $Na_2B_4O_7$) should be used as the buffering agent because other buffering agents may hinder identification by leaving a precipitate on body tissues and setae.

A 10 percent buffered formalin solution is prepared by adding 4 oz of borax to each gallon of concentrated formalin (i.e., a 40 percent solution of formaldehyde in water). This amount will be in excess, so use the clear supernatant when making seawater dilutions. Dilute the concentrate to a ratio of one part concentrated formalin to nine parts seawater. Seawater will further buffer the solution. Seawater also makes the fixative isotonic with the tissues of the animals, thereby decreasing the potential for animal tissues to swell and break apart, as often happens with freshwater dilutions of formalin.

washed using a combination of these techniques. For all methods, it is imperative that the samples be washed gently to minimize specimen damage. A few minutes of extra care in the field can save hours of time for the taxonomist, and will result in a better data set.

For many surveys, it is easiest to wash the samples from above with a gentle spray, because efficient, easy-to-use gear may be constructed to hold the sampler and sieve boxes. MTS recommends the use of a high volume low pressure seawater pump to get filtered seawater for sieving purposes.

All wash water is to be filtered (using a cartridge-filter system) or screened through a mesh with openings less than one half the size of those used in the survey, so as not to introduce planktonic or bentho-pelagic organisms into the samples. Failure to screen in this way can result in increased sorting time. It can also compromise the quality of the resulting data, because it is impossible to distinguish bentho-pelagic organisms caught by the grab from those entrained in the wash water. Never use fresh water to wash marine samples as destruction of soft-bodied organisms will occur through the disparity in osmotic potentials.

Once sieving is completed, the screen box should be held at an angle and the remaining material gently washed into one corner. The sample may then be transferred to a container for relaxation, if desired, or for immediate fixation, using as little seawater as possible. A permanent internal sample label is placed in the container at this time. If more than one screen fraction is generated, be sure to keep them separate throughout all phases of field and laboratory processing. Be sure to check the screen for organisms trapped in (or wound around) the mesh wires. If they cannot be dislodged with gentle water pressure, use a pair of jewelers forceps to remove them. Be careful not to damage the wire mesh. After the screen has been checked for remaining animals and sample removal is complete, back-wash the screen with a high-pressure spray to dislodge any sediment grains that may be caught in the mesh.

As mentioned earlier, a 10-15 percent solution of borax-buffered formalin usually is sufficient to fix benthic organisms. However, samples containing large amounts of fine-grained sediments, peat, or woody plant material may require higher concentrations. The volume of fixative should be at least twice the volume occupied by the sample. The formalin solution should be added to the sample container until it is completely filled. This will minimize abrasion during shipping and handling. If the sample volume exceeds one half of the container volume, more than one container should be used. Use of multiple containers for single samples should be recorded on the log sheet.

MTS recommends that fresh fixative be prepared prior to each sampling excursion, as formalin will eventually consume all the buffering capacity of the borax. Formalin solution of any strength should not be exposed to freezing temperatures, because the formaldehyde polymers will degrade into paraformaldehyde and the solution will have to be discarded.

Rose Bengal Preparation--

If staining is to be used to aid in sorting, rose bengal may be added to the samples either as a powder or a solution. Both are effective. However, it is easier to use a solution. A rose bengal concentration of 4 g/L of concentrated formalin commonly is used (Eleftheriou and Holme 1984).

Sample containers--

Samples can be stored in a variety of containers including glass or plastic jars, and plastic or muslin bags. If jars are used, plastic lids are preferable to metal lids because formalin corrodes metal. If glass jars are used, extra care should be taken when handling, shipping, and storing them to prevent breakage. If plastic or muslin bags are used, extra care should be taken to prevent them from tearing. MTS prefers the use of plastic jars with plastic or plastic lined lids.

In general, a single 1 or 2 quart container is large enough to hold a sieved sample from a 0.1 m^2 sampler. However, more or larger containers may be required if large quantities of gravel, peat, wood chips, or other large items occur in the sample.

Labels--

MTS field and laboratory people use a complete label inside each sample container, as well as on the side of each container. An abbreviated label is placed on the caps of jars to identify them when in shipping or storage cases. All MTS labels are made of waterproof rag paper and the external labels are gummed. External labels may be filled out using waterproof ink, but internal labels must be filled out using only a #2 pencil.

Processing

MTS highly recommends that the entire sample be sieved for benthic infaunal analyses. If samples are needed for physical or chemical analyses, they should be taken from a separate sample.

After qualitative characteristics of the sample have been recorded, sediments are washed on the designated sieve(s). MTS recommends the use of a 0.5 mm sieve for macro benthic work. Sediment adhering to the outside of the sampler should not be mixed with the sample. When being sieved, sediments can be gently sprayed with water from above, gently agitated by hand in a washtub of water (in an up-and-down, not swirling, motion), or

Analytical Procedures

Transfer to Alcohol--

Samples are to remain in the formalin-seawater solution for a minimum of 24 hours to allow proper fixation (Fauchald 1977). A maximum fixation period of 10 days is recommended by MTS to reduce the risk of decalcifying molluscs and echinoderms. After fixation, the samples should be washed (i.e., rescreened) on a sieve with mesh openings of at least half the size of those used in the field. The smaller screen size ensures that the specimens collected in the field will be retained in the sample regardless of shrinkage or breakage resulting from contact with the formalin. MTS has found it desirable to wash the formalin from the samples as soon as possible after the initial 24 hours because the buffering capacity of the borax in the formalin solution decreases continually.

If the sample consists of multiple containers, all containers are located prior to rescreening and washed at the same time. The contents of each container are carefully poured onto the appropriately sized screen. The container should be rinsed to remove adhering organic material, sediment, or organisms. The screen is not to be filled more than half full to avoid spilling or splashing the sample.

There are several acceptable methods for rinsing formalin from a sample. The MTS recommended method is to gently flush the sample with large quantities of fresh water from a low-pressure faucet or hose, being careful not to splash any sample material. A second method is to partly immerse the sieve in a plastic tub filled with fresh water and wash the sample by moving the sieve in an up and down motion. Care must be taken not to let the water rise above the top level of the sieve. Allow the rinse water to completely drain from the sieve and lightly rinse the sample with a solution of 70 percent alcohol from a squirt bottle. Carefully wash the sample material into a sample jar filling it no more than three-quarters full. Rinse the last bit of material into the jar using the squirt bottle of alcohol. Fill the jar to the top with the 70 percent alcohol solution and screw the lid on tightly. Gently shake and invert the jar several times to ensure proper mixing.

Each jar should have one internal label and two external labels. The internal label should be made of waterproof, 100 percent rag paper and filled out using a #2 pencil. One label is attached to the side of the jar and the second should be attached to the lid of the jar. All three labels will include all information recorded on the field data tag, plus all other information needed to ensure proper identification of the sample.

After fixative has been added to a sample container, it is critical that the contents be mixed adequately. This usually can be accomplished by inverting the container several times. After mixing, sample containers are to be placed in protective containers for storage and transport to the laboratory. After being stored for approximately 1 h, samples should be inverted several times again to ensure adequate mixing.

On board ship, samples should be stored so as to minimize exposure to sunlight and temperature extremes. They should also be stored in a stable part of the ship to minimize agitation.

Laboratory Procedures

Equipment and Supplies--

The MTS laboratory is equipped with both Zeiss and Wild stereo dissection and Zeiss compound microscopes. Magnifying lamps are also available for sorting samples. Compound microscopes are capable of magnifications up to 1,000 power. The optics of these microscopes are of the highest quality. Other MTS laboratory supplies include jewelers forceps, fine scissors, small scalpels, fine needles, flat and depression microscope slides, cover slips, small dissection trays, immersion oil, glycerol alcohol (half glycerol and half 70 percent alcohol), numerical counters, fiberoptic light sources and miscellaneous glass and plastic ware.

Preservative Preparation--

After the specimens are fixed, alcohol is used as a long-term preservative. Either 70 percent ethanol (v/v) in water or 70 percent isopropanol (v/v) are used (Fauchald 1977). Specimens preserved in isopropanol are unsuitable for histological examination. If future studies of anatomy or reproductive biology are anticipated, ethanol will be used.

To prepare 1 L of a 70 percent solution of either alcohol, add 263 mL of distilled water to 737 mL of 95 percent alcohol solution.

Use of the 70 percent alcohol/30 percent water solution is adequate for the preservation of most infaunal organisms (Fauchald 1977; Eleftheriou and Holme 1984). For long-term storage of crustaceans, however, it is recommended that glycerine be substituted for some of the water. The glycerine helps keep the exoskeletons supple, thereby facilitating examination and manipulation. This is especially critical for crustaceans archived in the reference collection (see below). An appropriate alcohol-glycerine solution would be 70 percent alcohol, 25 percent water, and 5 percent glycerine (Eleftheriou and Holme 1984).

Each sample will be sorted by only one person. At a minimum, organisms should be sorted into the following major taxonomic groups: Annelida, Arthropoda, Mollusca, Echinodermata, and miscellaneous phyla (combined). All organisms will be placed in large vials containing a 70 percent alcohol solution. Each vial containing a major taxonomic group should have an internal label listing the survey name, station designation, water depth, date sampled, and field screen size. All vials from the same sample will be stored in a common container and immersed in the 70 percent alcohol solution. To reduce evaporation of alcohol, lids will be sealed with plastic electrical tape.

Biomass Determination--

MTS is equipped to to perform wet weight biomass. When required, biomass estimates for the major taxonomic groups will be made prior to identifying the organisms to the species level. It is recommended, however, that taxonomists examine the major taxonomic groups before biomass measurements are made, to ensure that sorters have correctly grouped all individuals and fragments and that the remains of dead organisms (e.g., empty mollusc shells) are not included. Biomass will be estimated to the nearest 0.1 g (wet weight). All specimens within a major group will be composited for biomass analyses: Annelida (principally polychaete worms), Mollusca (principally bivalves, gastropods and aplacophorans), Arthropoda (principally crustaceans), Echinodermata (principally asteroids, ophiuroids, echinoids, and holothuroids), and miscellaneous taxa (combined). These five categories generally are adequate to characterize the standing stocks of the major infaunal groups. They also are sufficiently distinct from each other to permit proper assignment of fragments to each of the groups. All fragments will be placed in their respective major taxonomic groups prior to weighing.

There are several major problems associated with the collection and interpretation of biomass information. Some taxa lose weight when immersed in preservative fluids, while others gain weight (Howmiller 1972; Lappalainen and Kangas 1975; Wiederholm and Eriksson 1977; Mills et al. 1982). For this reason, the most accurate biomass estimates are performed on live material. However, it is rarely practical to sort and weight live specimens. Accurate measurements of biomass may be compromised further by evaporation from the specimens while they are on the balance. Lastly, biomass measurements are only estimates of standing crop. They do not reflect estimates of production because all organisms are treated in the same manner whether they are large and long-lived, or small and short-lived. Because of these problems, biomass measurements should be interpreted carefully.

All jars of a given sample are kept together (if more than one), and all replicate samples from a given station are stored together. As the samples are shelved prior to sorting, each will be cross-referenced to the field log sheet. At this point the sample custodian will date and initial the rescreening section of the sample tracking form for each station. Washed samples are stored in an upright secure position at a cool temperature, and away from direct sunlight. Samples are periodically curated.

Sample Sorting--

MTS uses several techniques to sort organisms from sediment. The most common technique involves placing a small amount of the sample into a glass or plastic grided petri dish and using a pair of jewelers forceps to sort through the sample in a systematic manner, removing each organism. This entire process is done while viewing the sample through a 10 power dissecting microscope or a magnifying lamp. Care must be taken that enough liquid is present in the petri dish to completely cover the sample to avoid reflections from the sediment/liquid interface which will cause distortion in the field of view. Each petri dish of material should be sorted twice to be sure that all organisms are removed.

A second sorting technique is a flotation method, which was found to be particularly effective when the sediment residue is primarily coarse sediment grains containing small amounts of organic matter (e.g., wood fragments, leaf debris, sewage sludge). The sample is first washed with fresh water in a large flat tray. The less dense material that becomes suspended in the fresh water (organic material, arthropods, and most soft-bodied organisms) is carefully poured into a sieve, and is sorted using the standard technique described above. The remaining material is covered with liquid and sorted using a 5 power self-illuminated lens. Organisms remaining in this portion of the sample generally include molluscs and some tube-dwelling or encrusting organisms that are associated with sand grains. Because it is difficult to see extremely small organisms with the 5 power lens, the sorter must remove all molluscs and polychaete tube fragments for closer inspection. All material collected from this portion is then placed into a labeled sample jar and viewed under a 10 power dissecting microscope to remove organisms from tubes and to ensure that the molluscs were alive when captured.

Whichever technique is used, the sorter is exposed to alcohol fumes. Because these fumes can be irritating to some people, the sorting process can be done using fresh water. However, as each portion of the sample is sorted, it should be drained and returned to the alcohol solution immediately.

Each taxonomist will record the initial identifications and counts in a notebook, which should also include notes and comments on the organisms in each sample. Upon completion of the sample, the data will be transferred to the sample data sheets and double-checked. The taxonomist will then sign and date the sample data sheet. All notebooks will be kept in the laboratory at all times so the laboratory supervisor can check questionable identifications and follow the progress of each sample.

QA/QC Procedures

Calibration and Preventive Maintenance

To make taxonomic identifications consistent within a given laboratory, and with the identifications of other regional laboratories voucher and reference collections will be used where available. At least three individuals of each taxon should be sent for verification to recognized experts. The verified specimens should then be placed in a permanent reference collection. Continued collection of a verified species does not require additional expert verification, because the reference collection can be used to confirm the identification. Participation of the laboratory staff in a regional taxonomic standardization program (if available) is recommended, to ensure regional consistency and accuracy of identifications. All members of the MTS taxonomic team belong to the Southern California Association of Invertebrate Taxonomists, however, due to travel distance, personal participation is not practiced.

All specimens in the reference collection will be held in labeled vials that are segregated by species and sample number. More than one specimen may be placed in each vial. The labels placed in these vials will be the same as those used for specimens in the sample jars. It is important to complete these labels, because future workers may not be familiar with the survey, station locations, and other details of the work in progress. In addition, the reverse side of the label should contain information about the confirmation of the identification by experts in museums or other institutions (if appropriate). Such information would include the name and institution of the outside expert, and date of verification. All vials for a given species should be placed in a single jar filled with alcohol. To reduce evaporation of alcohol, the lids of the jars will be sealed with plastic electrical tape wrapped in a clockwise direction. Reference specimens will be archived alphabetically within major taxonomic groups. A listing of each species name, the name and affiliation of the person who verified the identification, the location of the individual specimen in the museum, the status of the sample if it has been loaned to outside experts, and references to pertinent literature will be maintained by the MTS laboratory taxonomists.

Several methods of measuring biomass are possible. One technique is to estimate the difference in weight of a tared beaker filled with preservative before and after organisms are placed in the beaker. The individual organisms are not blotted prior to weighing, and as few individuals as possible are transferred to the weighing container. These procedures minimize the transfer of fluids held within a pile of individuals. This technique can be used for preserved or live animals, and appears to introduce the least amount of variation into the weighing process.

A second technique for biomass determination consists of air-drying the organisms on absorbent paper for a specific length of time (e.g., 5 min). Because 70 percent ethanol is volatile, small variations in drying time may increase the errors associated with the weight measurements. A container open at one end and covered at the other end with a 0.25-mm mesh screen (maximum mesh opening) can be used to hold the organisms for weighing. After the tare weight of the container is measured, the animals are carefully placed into the container. The container with organisms is then placed on a paper towel and allowed to air dry for exactly 5 minutes prior to weighing. The weight of the organisms is obtained by subtracting the weight of the container with the organisms from the tare weight of the container. Extremely large organisms (e.g., large molluscs or asteroids) should be weighed individually.

Taxonomic Identification--

After biomass estimates are completed, identification and counting of the organisms may begin. Unless otherwise specified, identifications will be to the lowest taxonomic level possible, usually the species level. For incomplete specimens only the anterior end is counted. All identifications should be made using binocular dissecting or compound microscopes. If possible, at least two pieces of literature will be used for each species identification. Moreover, each species identification will be checked against a reference specimen from a verified reference collection if one exists (see QA/QC Procedures).

After completing taxonomic identifications, all organisms will be placed in vials containing 70 percent alcohol. All vials for a single sample will be stored in common jars and immersed in 70 percent alcohol. Each vial will contain an internal label with the following information: survey name, station number, replicate number, collection gear, water depth, and date of collection. Any specimens removed from the sample jar and placed in the reference collection will be so noted (species, number) on the sample identification sheet.

Corrective Action

Following QA/QC procedures discussed earlier, each 20 percent sample aliquot is checked for complete or nearly complete removal of organisms. Thus, each sample elicits a decision concerning a possible re-sort. When a sample is found that does not meet the recommended 95 percent removal criterion (see Data Quality and Reporting Requirements below), it will be re-sorted.

When a taxonomic error or inconsistency is found, it is MTS policy to trace all of the work of the taxonomist responsible for the error, so as to identify those samples into which the specific error or inconsistency may have been introduced. This process can be very time-consuming. However upon completion of all taxonomic work, few (if any) taxonomic errors or inconsistencies remain in the data set. Avoiding errors and inconsistencies through the constant interchange of information and ideas among taxonomists is the best way to minimize lost time due to faulty identification.

Data Quality and Reporting Requirements

At MTS a sample sorting efficiency of 95 percent of total number of individuals is considered acceptable. That is, no more than five percent of the organisms in a given sample are missed by the sorter. Similarly, species identifications by each taxonomist can reasonably be expected to be accurate for at least 95 percent of total number of species. Unless otherwise specified, all organisms will be identified to the lowest possible taxon; to species level whenever possible. In cases where the identity of a species is uncertain, a species number is used (e.g., Macoma sp. 1, Macoma sp. 2). Numerical designations must be consistent throughout each study. To facilitate comparability among different studies, the distinguishing characteristics of each unidentified species will be recorded. Data for each replicate sample is reported as numbers of individuals per sample for each species and as biomass (nearest 0.1 g wet weight per sample) for each major taxonomic group.

Reference specimens are invaluable, and will be retained in the MTS laboratory, in the offices of the funding agencies, or at a museum with long-term storage capabilities. In no instance should this portion of the collection be destroyed.

Quality Control Checks

MTS quality control procedure recommends that at least 20 percent of each sample be re-sorted for QA/QC purposes. Re-sorting is the examination of a sample that has been sorted once and is considered free of organisms. The 20 percent aliquot should be taken after the entire sample has been spread out in a pan or tray. It is critical that the aliquot be a representative subsample of the total sample. Care is taken to include any organisms that may be floating in the preservative. Re-sorting will be conducted using a dissection microscope capable of magnification to 25 power. A partial re-sorting of every sample will ensure that all gross sorting errors are detected. In addition, it will give added incentive to sorters to process every sample accurately. Re-sorting will be conducted by an individual other than the one who sorted the original sample.

In addition to efficient sample sorting, consistent identification of organisms among individuals and among sampling programs is critical to the collection of high quality data. Consistent identifications are achieved by implementing the procedures discussed below and by maintaining informal, but constant, interaction among the taxonomists working on each major group. One important procedure at MTS is to verify identifications by comparison with the reference collection specimens. To ensure that identifications are correct and consistent, 5 percent of all samples identified by one taxonomist should be re-identified by another taxonomist who is also qualified to identify organisms in that major taxonomic group. MTS uses the following specialists to verify identifications (see attached sheet). It is the duty of the senior MTS taxonomist to decide upon the proper identification(s). The senior taxonomist may also decide whether the taxonomic level to which a given organism is identified is appropriate. If it is not, the senior taxonomist may decide to drop back to a higher taxonomic level, or to further refine the taxonomy of that group through additional study.

When all identifications and QA/QC procedures are completed, the jars containing the vials of identified species are topped off with 5 percent glycerine/70 percent alcohol. The lids are then sealed tightly with black electrical tape to prevent evaporation. All sample jars are be placed in containers filled with 70 percent alcohol for long term storage. The containers are fitted with a tightly sealed lid, and electrical tape is again used to seal the joints. Each container is labeled clearly with the survey name, date, and number and type of samples within it.

APPENDIX D

RAW DATA REQUIREMENTS FOR DAIS

SPREADSHEET A

Header:

1. Survey (project) name
2. Tracking number (Corps will provide)
3. Section 10/404 Permit application number
4. Applicant name
5. Date spreadsheet prepared

Sampling Stations:

6. Station numbers
7. Latitudes and Longitudes (min. precision = 0.1")
8. Horizontal datum (NAD 1927 or 1983)
9. Water Depth in feet corrected to MLLW
10. Control/Reference Station Names

Lab Samples:

11. Lab Sample Codes
12. Sampling stations and depths comprising each sample
13. Earliest Sampling Date
14 .Subarea numbers and ranks.

SPREADSHEET B

Conventional Chemistry (Total Solids, Total Volatile Solids, Total Organic Carbon, Total Sulfides, and Ammonia):

1. Lab Names
2. Batch Composition
3. Preparation and analysis codes
4. Replicate numbers
5. Analyte Measurements and Qualifiers
6. Units and Method Blank Units (i.e. %, mg/kg...)
7. Method Blank results for TOC, Ammonia and Sulfides
8. Analysis Dates
9. TOC CRM 95% Confidence Interval
10. TOC CRM analysis results

Grain Size Analysis:

11. Fine-grain analysis method (pipette or hydrometer)

Grain Size Analysis:

11. Fine-grain analysis method (pipette or hydrometer)
12. Batch Composition
Replicate numbers
13. Analysis Dates
14. Grain Size intervals
15. Percent falling within each interval

SPREADSHEET C

Sample Preparation Data and Sample Weights:

1. Extraction/Preparation Group Names
2. Extract/Preparation Codes
3. Extraction/preparation dates
4. Method Blank start dates
5. Surrogates used for each chemical group
6. Lab names
7. Batch composition (including reference materials)
8. Replicate numbers
9. Sample Weights

*NOTE: It is critical for the sample weight to be recorded for each sample taken in the particular extraction/preparation group (including the CRMs, RMs, and matrix spikes). This is a key field in the DAIS database and will drive the automated input screen displays.

Analysis Data:

10. For each chemical-of-concern there is a common list of needed data:
    -extract/prep group number
    -analysis method code
    -units (dry weight basis)
    -blank units
11. Sample analysis information includes the following:
    -replicate number
    -analysis date
    -concentration
    -data qualifier (if necessary)

12. Analysis data are needed for the following:
    -test sediments
    -reference materials
    -method blanks

-matrix
-spiked samples

13. Matrix spikes must be reported on a sample-specific dry-weight-normalized basis

Surrogate Recoveries:

14. Analysis method codes
15. Replicate numbers
16. Analysis dates
17. Recovery for test sediments, method blanks, reference materials and matrix-spiked samples.

SPREADSHEET D

Amphipod Mortality and Emergence:

1. Species name
2. NODC code
3. Exposure Time
4. Lab Name
5. Lab Sample Codes
6. Batch Composition
7. Start Dates
8. Daily Emergence Counts (for 10 days)
9. Number of Survivors
10. Number Failing To Reburow

Amphipod Bioassay Positive Control:

11. Toxicant used
12. Exposure Time
13. LC50 Method of calculation
14. Batch numbers
15. Start Dates
16. Toxicant concentrations
17. Percent survival at each concentration
18. LC50

Amphipod Bioassay Water Quality:

19. Methods used:
    -Dissolved Oxygen
    -Water Salinity

-Interstitial Water Salinity
-Ammonia
-Sulfide

20. Batch numbers
21. Daily Temperature
22. Daily pH
23. Daily DO (mg/L)
24. Daily Water Salinity (ppt)
25. Initial Interstitial Salinity (ppt)
26. Initial and Final Sulfide (mg/L)
27. Initial and Final Ammonia (mg/L)

Sediment Larvae Mortality and Abnormality:

28. Species Name
29. NODC Code
30. Lab Name
31. Inoculation Time (hrs.) = the length of time after the sediment is placed into the beaker and before the organisms are added.
32. Exposure Time (hrs.)
33. Test Beaker Volume (ml)
34. Stocking Density (eggs/ml) for each batch = concentration of eggs in the beaker from which all test beakers are stocked
35. Stocking Aliquot Size (ml) = the volume taken from the fertilization beaker to stock each of the test beakers.

Sediment Larval Test Results:

36. Batch composition
37. Start Dates

For initial counts, seawater controls, test and reference sediments:

38. Number of aliquots counted
39. Aliquot Size (ml)
40. Number of Normal per aliquot
41. Number of Abnormal per aliquot

Sediment Larval Bioassay Positive Control (% Survival):

42. Toxicant
43. Exposure Time (hrs)
44. LC50 Method of Calculation
45. Batch numbers

46. Start Dates
47. Toxicant Concentrations
48. % Survival for each concentration

Sediment Larval Bioassay Positive Control (% Abnormality):

49. Same as #42-48, except for 44. which should be - EC50 Method of Calculation

Sediment Larval Bioassay Water Quality:

50. See #19-27, exclude Interstitial Water Salinity

Juvenile Infaunal Species Bioassay Mortality and Growth

51. Species Name
52. NODC code
53. Lab name
54. Starting Age (in days post emergence)

20-day growth test only:

55. Food Type
56. Feeding Interval (hrs.) = the time between feedings
57. Feeding Quantity (mg dry weight/individual/feeding event)

Juvenile infaunal species
beginning biomass

58. Batch and Rep number
59. Analysis Date
60. Number of Organisms Weighed
61. Total Start Weight (mg/dry)
62. Organisms Depurated (yes or no)
63. Total End Weight of Survivors (mg dry)

Juvenile Infaunal Species Mortality:

64. Batch number and start date
65. Number of Organisms Beginning
66. Number of Survivors
    Initial weight of organisms
    Final weight of organisms

Juvenile Infaunal Species Bioassay Positive Control (% Survival):

67. See #42-48
68. LC50 mg/L

Juvenile Infaunal Species Bioassay Water Quality:

69. See # 19-27

Microtox Bioassay:

70. Lab Name
71. Batch Composition
72. Extraction Time
73. Extraction Date
74. Analysis Date
75. Analysis Time
76. Extract Dilutions
77. Initial and Final illumination values for rep 1 and rep 2 for each dilution (including the blank)
78. Initial and Final illumination values for five replicates at the highest concentration

Microtox Bioassay Positive Control:

79. Toxicant
80. EC50 Method of calculation
81. See #72-78
82. EC50 %