APPENDIX I

ADDITIONAL CONTAMINANTS OF CONCERN AND REPORTING LIMITS

TABLE I-1. Additional chemical contaminants[1], recommended methods, and reporting limits routinely analyzed in sediments and tissue

Contaminant	Analytical Method(s)	Sediment Reporting Limit (dry wt)	Tissue Reporting 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
Miscellaneous			
Cyanide	9010, 9012	2.0 ppm	1.0 ppm
Acid Volatile Sulfides	Allen et al., 1991	0.01 umol/g	N/A
Organotins	Rice et al., 1987 Uhler & Durrel, 1989	10 ppb	10 ppb
BASE/NEUTRALS			
Aromatic Hydrocarbons	8270[2]	20 ppb[2]	20 ppb[2]
Biphenyl			
Benzo(e)pyrene			
2-6-Dimethylnaphthalene			
1-Methylphenanthrene			
1-Methylnaphthalene			
2-Methylnaphthalene			
Perylene			
Phthalates	1625C, 3540, 82502	50 ppb[2]	20 ppb[2]
Dimethylphthalate			
Diethylphthalate			
Di-n-butylphthalate			
Butyl benzyl phthalate			
Bis(2-ethylhexyl) phthalate			
Di-octyl phthalate			

TABLE I-1 (continued). Additional chemical contaminants[1], recommended methods, and reporting limits routinely analyzed in sediments and tissue

Contaminant	Analytical Method(s)	Sediment Reporting Limit (dry wt)	Tissue Reporting Limit (wet wt)
Dioxins/dibenzofurans	8290, 1613 (pptr)	(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
WHO PCB Congeners	1668[2]	0.25 ppb[2]	0.5 ppb[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			

1 Chemical constituents on this optional list would be stipulated by the CENAE 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.

APPENDIX II

QUALITY CONTROL SUMMARY TABLES

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 reporting limits 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 12 months?	
15. Were the SRM/CRM analyses within acceptance criteria?	

Table II-1 (continued): Completeness Checklist

Quality Assurance/Quality Control Questions	Yes/No? Comments?
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?	

Table II-2: Quality Control Summary for Analyses of Polyaromatic Hydrocarbons (PAHs) and other base-neutrals in Sediment and Tissue Matrices

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 12 month period (see Section 5.2 for MDL procedure)			In Data Package
Calibration Verification (Second Source)	Once, after initial calibration (80 to 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 > RL			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 to 120%; RPD <30%)			In Data Package

Table II-2 (continued): Quality Control Summary for Analyses of Polyaromatic Hydrocarbons (PAHs) and other base-neutrals in Sediment and Tissue Matrices

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)
Analytical Replicates	Analyze one sample in duplicate for each group of field samples (RPD < 30%)			In Data Package
Surrogate Recoveries	Calculate % recovery (30 to 150% recovery)			In Data Package
Internal Standard Areas	Within 50 to 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.

Table II-3: Quality Control Summary for Analyses of Pesticides in Sediment, Tissue and Water Matrices

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 12 month period (see Section 5.2 for MDL procedure)			In Data Package
Calibration Verification (Second Source)	Once, after initial calibration (80 to 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 > RL			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 to 120%; RPD <30%)			In Data Package

Table II-3 (continued): Quality Control Summary for Analyses of Pesticides in Sediment, Tissue and Water Matrices

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)
Analytical Replicates	Analyze one sample in duplicate for each group of field samples (RPD < 30%)			In Data Package
Surrogate Recoveries	Calculate % recovery (30 to 150% recovery)			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 for Analyses of Polychorinated Biphenyls (PCB congeners) in Sediment, Tissue and Water Matrices

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 12 month period (see Section 5.2 for MDL procedure)			In Data Package
Calibration Verification (Second Source)	Once, after initial calibration. (80 to 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 > RL			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 to 120%; RPD <30%)			In Data Package

Table II-4 (continued): Quality Control Summary for Analyses of Polychorinated Biphenyls (PCB congeners) in Sediment, Tissue and Water Matrices

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)
Analytical Replicates	Analyze one sample in duplicate for each group of field samples (RPD < 30%)			In Data Package
Surrogate Recoveries	Calculate % recovery (30 to 150% recovery)			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 for Analyses of Metals in Sediments, Tissue and Water Matrices

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 12 month period (see Section 5.2 for MDL procedure)			In Data Package
Initial Calibration Verification/ Continuing Calibration Verification	Hg: 80 to 120% recovery Other metals: 90 to 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 > RL			In Data Package

Sample Spike/ Sample Duplicate	One set per group of field samples. Must contain all target analytes. Recovery Limits (75 to 125%; RPD < 20% or < 35%)			In Data Package

Table II-5 (continued): Quality Control Summary for Analyses of Metals in Sediments, Tissue and Water Matrices

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)
Analytical Replicates	Analyze one sample in duplicate for each group of field samples (RPD < 30%)			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-6: Quality Control Summary for Analyses of other Organic Chemicals not listed in Sediment, Tissue and Water Matrices

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)
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 12 month period (see Section 5.2 for MDL procedure)			In Data Package
Calibration Verification (Second Source)	Once, after initial calibration (80 to 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 > RL			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 to 120%; RPD <30%)			In Data Package

Table II-6 (continued): Quality Control Summary for Analyses of other Organic Chemicals not listed in Sediment, Tissue and Water Matrices

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 < 30%)			In Data Package
Surrogate Recoveries	Calculate % recovery (30 to 150% recovery)			In Data Package
Internal Standard Areas (if applicable)	Within 50 to 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.

Table II-7: Quality Control Summary for Analyses of Sediment Grain Size and Total Organic Carbon

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)
Grain Size: 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 (RPD < 30%)			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-8: Quality Control Summary for Biological Toxicity Testing only

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., Ammonia (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: Control mortality Control abnormality	 $\leq$ 10% mean $\leq$ 30% mussel/oyster; $\leq$ 40% clam larvae, $\leq$ 30% sea urchin larvae			In Data Package
Sediment toxicity test: Control mortality Compliance with applicable test acceptability requirements in Table 11.3 (EPA 1994a)	 $\leq$ 10% mean (no chamber > 20%) 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.

APPENDIX III

FORMS FOR ATTERBERG LIMITS

*

LIQUID AND PLASTIC LIMIT TESTS
For use of this form, see EM 1110-2-1906.

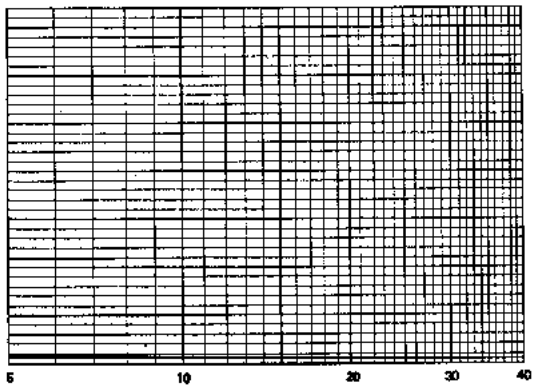
DATE _____

PROJECT _____

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 _____

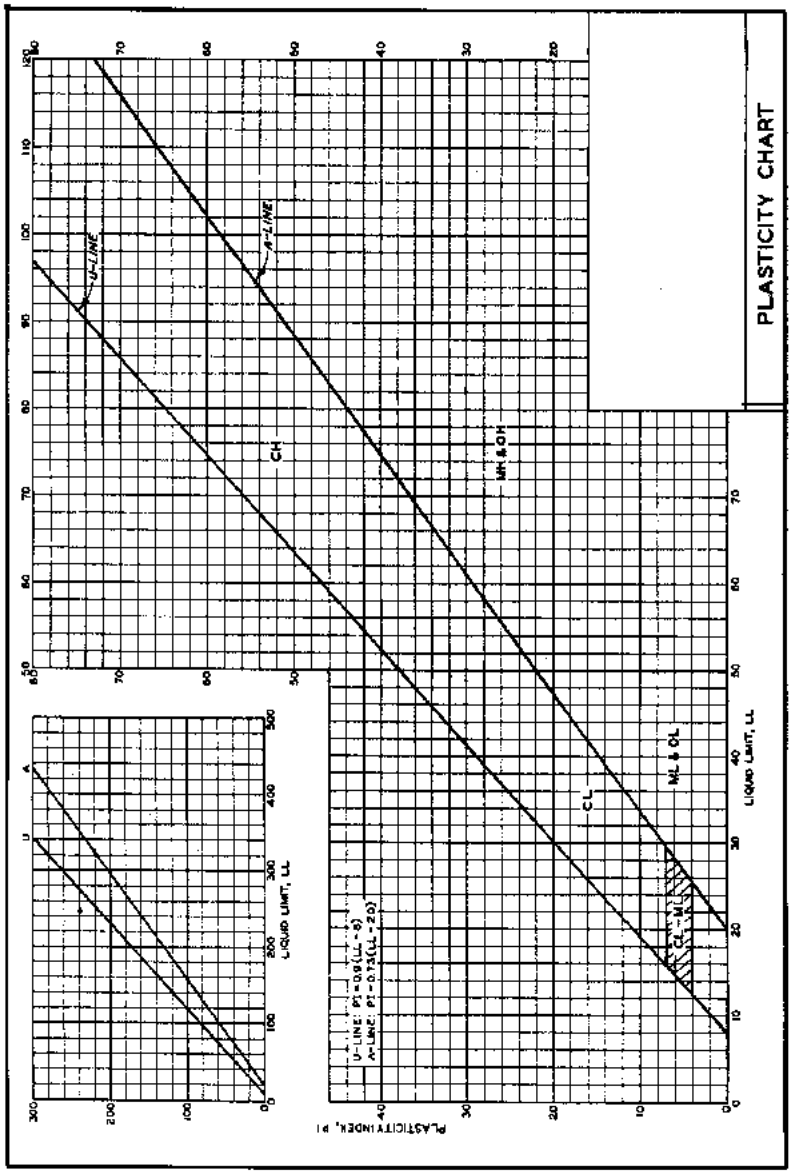
TECHNICIAN _____ COMPUTED BY _____ CHECKED BY _____

ENG FORM 3838 JUN 85

GPO : 1983 O -407-134

PLATE II-1

*


PLASTICITY CHART
U-LINE
A-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)
TRANSLUCENT
ENG FRM 4334 (EM 1110-2-1906)
JUN 3

APPENDIX IV

AED LABORATORY OPERATING PROCEDURE FOR OPERATION OF HIGH VOLUME WATER SAMPLER

AED LABORATORY OPERATING PROCEDURE OPERATION OF HIGH VOLUME WATER SAMPLER FOR EXTRACTION OF NON-IONIC ORGANIC ANALYTES

POINT OF CONTACT:

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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.

APPENDIX V

AED LABORATORY OPERATING PROCEDURE FOR SEA URCHIN LARVAL DEVELOPMENT TEST

AED LABORATORY OPERATING PROCEDURE CONDUCTING THE SEA URCHIN LARVAL DEVELOPMENT TEST

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 0.000l 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, l0-l000 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®, 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 l0 (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 $\pm$ 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 $\pm$ 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 l 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 $l0^7$ 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 $\pm$ 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 l 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 $\pm$ 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 l0% 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).

APPENDIX VI

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

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

REFERENCE:

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

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

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

REFERENCE:

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

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

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

* - Protocol dependent

REFERENCE:

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

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

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

REFERENCE:

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

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

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20°C
4. Salinity:	20 to 35 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	Continuous Light
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 950 mL
10. Sediment depth:	4 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Immature amphipods, or mature females only
13. No. of organisms per test chamber:	20 to 30
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100 to 150
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	Trickle-flow (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared using Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

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

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

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

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

REFERENCE:

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

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

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

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

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

REFERENCE:

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

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

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE 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 *Mysidopsis bahia* *Mysidopsis bigelowi* *Mysidopsis almyra* 20±1°C for *Neomysis americana* 12±1°C for *Holmesimysis costata*
4. Salinity:	25-30 ‰ ±10% except for *Holmesimysis costata* which is to be 32-34 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/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: *Mysidopsis bahia* *Mysidopsis bigelowi* *Mysidopsis almyra* *Neomysis americana* and DO> 60% saturation for: *Holmesimysis costata* (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artificial seawater prepared with Millipore MILLI-Q® or equivalent or deionized water
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A

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

REFERENCE:

Modified from:

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

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

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

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

[t] Slurry of Yeast, YCT, Trout chow.

REFERENCES:

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

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

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

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE 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.

APPENDIX VII

PORE WATER COLLECTION PROCEDURE FOR AMMONIA MEASUREMENT

Pore Water Collection Procedure for Ammonia Measurement

Set up of surrogate (or "dummy") containers

Porewater ammonia measurements should be made in surrogate chambers (chambers with no animals added) for each homogenized sediment treatment level (control, reference, dredged material site). Total and un-ionized ammonia levels must be monitored in the pore-water on days 1, 3 (or 5) and 10 during the test. Therefore, three additional containers (one for each monitoring day) should be maintained for each sediment treatment (control, reference, dredged material site).

Collection of Pore water:

Interstitial pore water should be extracted by centrifuge using the methods described in Burgess et al. (1993) or in Ferretti et al. (2000). Here, up to 200 ml of sediment (typically 100 ml is sufficient) is placed in a 250 ml teflon centrifuge tube and centrifuged at 4°C for 60 minutes at 4,000 rpm (2520 G) or 30 minutes at 8000 rpm. In general, about 20 ml of interstitial water would be needed to measure ammonia with an ion-selective electrode. Ferretti (personal communication) observed that 100 ml of sediment is usually sufficient to capture 25 to 50 ml of pore water. Alternatively, interstitial pore water may be collected using peepers (see Section 6.2.1 of EPA 2001d).

Analysis of Ammonia:

Total and un-ionized 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). Acceptable detection limits are 0.1 mg/L. Un-ionized ammonia can be calculated using the dissociation model of Whitfield (1972) as programmed by Hampson (1977).

Bower, C.E. and T. Holm-Hansen. 1980. A Salicylate-hypochlorite Method for Determining Ammonia in Seawater. Can. J. Aquat. Sci. 37: 794-798.

Burgess, R.M., K.A. Schweitzer, R.A. McKinney, and D.K. Phelps.1993. Contaminated Marine sediments: Water column and Interstitial Toxic Effects. Environmental Toxicology and Chemistry12: 127-138.

Ferretti, J.A., D.F. Calesso, and T.R. Hermon. 2000. Evaluation of methods to remove ammonia interference in marine sediment toxicity tests. Environmental Toxicology and Chemistry19(8): 1935-1941.

Hampson, B.L. 1977. The Analysis of Ammonia in Polluted Sea Water. Water Research 11: 305-308.

Merks, A.G.A. 1975. Determination of Ammonia in Sea Water with an Ion-Selective Electrode. Netherlands J. Sea Res. 9: 371-375.

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

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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 Rubinstein (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)

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

hydrogen phthalate (pH 4.00) and (2) disodium hydrogen phosphate (pH 7.4). For overnight or weekend storage, the electrode was held in salt water, leaving the fill hole open. For daily use, the outer half-cell was filled with electrolyte to the fill hole and the electrode checked for stability. The electrode pair was calibrated once daily prior to measuring pH of samples; it was never recalibrated during a series of measurements. Following calibration, the electrode was soaked in sea water, of salinity similar to the sample, for at least 15 minutes to achieve chemical equilibrium and a steady state junction potential. When measuring pH, the sample was initially gently agitated or stirred to assure good mixing at the electrode tip, but without entraining air bubbles in the sample. Stirring was stopped to read the meter. The electrode was allowed to equilibrate so the change in meter reading was less than 0.02 pH unit/minute before recording. Following each measurement, the electrode was rinsed with sea water and placed in fresh sea water for the temporary storage between measurements. Additional suggestions to improve precision of saltwater pH measurements may be found in Zirno (1975), Grasshoff (1983), and Butler et al. (1985).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
27 TARZWELL DRIVE
NARRAGANSETT, RHODE ISLAND 02882

May 20, 1994

Subject: Mysid No Effect NH_3 Concentration for Lethality and pH Issues for Sediment Toxicity Test Protocols
From: Don C. Miller DCM
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

APPENDIX IX

AED LABORATORY OPERATING PROCEDURE FOR 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.