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.