APPENDIX E
SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR TIER III BIOASSAYS

Acute Toxicity
Water Column Tests

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 GRASS SHRIMP, *Palaemonetes* sp., ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	25±1°C
4. Salinity:	30-35 ‰ ±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:	1 L minimum
9. Test solution volume:	750 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	1-4 d from hatch
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:	None
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 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:	4 L per site minimum
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

Modified from the mysid acute toxicity water column test published in:

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

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR COMMERCIAL SHRIMP, *Penaeus* sp., ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	25±1°C
4. Salinity:	30-35 ‰ ±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:	80 L
9. Test solution volume:	60 L
10. Renewal of test solutions:	None
11. Age of test organisms:	8-10 d post larvae
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:	None
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 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:	20 L for site sediment
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

Modified from the mysid shrimp acute toxicity water column test published in:

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

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE CLADOCERANS, *Daphnia magna* AND *D. pulex,* 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:	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:	30 mL minimum
9. Test solution volume:	25 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	Less than 24 h old
12. No. organisms per test chamber:	5 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	25 minimum
15. Feeding regime:	Feed YCT* and *Selenastrum* while holding prior to the test; newly-released young should have food available a minimum of 2 h prior to use in a test; add 0.2 mL each of YCT and *Selenastrum* at -2 h and at 48 h.
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. 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
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

* Slurry of Yeast, Cereal flakes, Trout chow.

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 THE CLADOCERAN, *Ceriodaphnia dubia,* 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:	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:	30 mL minimum
9. Test solution volume:	15 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	Less than 24 h old
12. No. organisms per test chamber:	5 minimum
13. No. replicate chambers per concentration:	5 minimum
14. No. organisms per concentration:	25 minimum
15. Feeding regime:	Feed YCT* and *Selenastrum* while holding prior to the test: newly-released young should have food available a minimum of 2 h prior to use in a test: add 0.1 mL each of YCT and *Selenastrum* at -2 h and at 48 h
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. Dilution water:	Moderately hard synthetic water prepared using Millipore MILLI-Q® or equivalent or deionized water and reagent grade chemicals, or 20% DMW, receiving water, or synthetic water modified to reflect receiving water hardness
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

* Slurry of Yeast, Cereal flakes, Trout chow.

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 THE SPECKLED SANDDAB, *Citharichthys stigmaeus*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test Duration:	96 h
3. Temperature:	15±2°C
4. Salinity:	30±2 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 $\mu E/m^2/s$ (50-100 ft-c)
7. Photoperiod:	16L/8D
8. Test chamber size:	30 L
9. Test solution volume:	20 L
10. Renewal of test organisms:	None
11. Age of test organisms:	Juveniles ≤ 8 cm
12. No. organisms per test chamber:	10
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 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:	20 L for site sediment
24. Test acceptability criterion:	≥ 90% survival in controls

REFERENCE:

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

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

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

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR GRUNION, *Leuresthes tenuis*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	20 or 25±2°C
4. Salinity:	20-32 ‰ ±10%
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 $\mu E/m^2/s$ (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 organisms:	None
11. Age of test organisms:	9 - 14 d
12. No. organisms per test chamber:	10
13. No. of 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, artifical 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 requirments:	<8 wk (sediment); elutriates are to be used within 24 h of preparation
23. Sample volume required:	20 L for site sediment
24. Test acceptability criterion:	≥ 90% or greater survival in controls

REFERENCE:

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

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

and from personal communications with Dr. Doug Middaugh, EPA, ERL-Gulf Breeze, FL.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR FATHEAD MINNOW, *Pimephales promelas*, BLUEGILL SUNFISH, *Lepomis macrochirus*, AND CHANNEL CATFISH, *Ictalurus punctatus*, 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:	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:	250 mL minimum
9. Test solution volume:	200 mL minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	Fathead minnow - on order of 4 d; 24 h range in age. Sunfish and Catfish - on order of 30 d
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:	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
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:	4L per site minimum
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 RAINBOW TROUT, *Oncorhynchus mykiss*, ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	96 h
3. Temperature:	12±1°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: Light intensity should be raised gradually over a 15 min period at the beginning of the photoperiod, and lowered gradually at the end of the photoperiod, using a dimmer switch or other suitable device
8. Test chamber size:	5 L minimum, test chambers should be covered to prevent fish from jumping out
9. Test solution volume:	4 L minimum
10. Renewal of test solutions:	None
11. Age of test organisms:	15-30 d (after yolk sac absorption to 30 d)
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:	Feeding not required
16. Test chamber cleaning:	None
17. Test solution aeration:	If needed to maintain DO> 60% saturation (< 100 bubbles/min.)
18. 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
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:	20 L for site sediment
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 SEA URCHIN, *Strongylocentrotus purpuratus,* AND SAND DOLLAR, *Dendraster excentricus*, SPERM CELL ACUTE TOXICITY WATER COLUMN TESTS

1. Test type:	Static Non-renewal
2. Test duration:	80 minute (60 minute exposure plus 20 minute fertilization period)
3. Temperature:	12°C
4. Salinity:	30±2 ‰
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:	Test tubes 16 x 100 or 125 mm
9. Test solution volume:	5 mL
10. Renewal of test solutions:	None
11. Age of test organisms:	Fresh eggs and sperm
12. No. organisms per test chamber:	560,000 sperm/1,120 eggs (100 eggs observed)
13. No. replicate chambers per concentration:	3 minimum
14. No. organisms per concentration:	300 eggs observed per concentration
15. Feeding regime:	None
16. Test chamber cleaning:	None
17. Test solution aeration:	None
18. Dilution water:	Filtered (0.45 µm): 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:	Egg fertilization percentage
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:	≥ 50% control fertilization, sperm:egg ratio between 250:1 and 1,000:1

REFERENCE:

Dinnel, P.A., Q.J. Stober, S.C. Crumley and R.E. Nakatani. 1982. Development of a sperm cell toxicity test for marine waters. Pp. 82-98 In: *Aquatic Toxicity and Hazard Assessment. Fifth Conference.* J.G. Pearson, R.B. Foster, and W.E. Bishop (Eds.). ASTM STP 766. American Society for Testing and Materials, Philadelphia, PA.

Acute Toxicity Sediment Tests

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, *Rhepoxynius abronius*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	15 ±3°C
4. Salinity:	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 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.

* 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, *Grandidierella japonica*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	15 - 19 ±3°C
4. Salinity:	30 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:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Immature amphipods 3 - 6 mm, no females carrying embryos
13. No. of organisms per test chamber:	20
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	100
16. Feeding regime:	Suspension of finely ground Tetramin and the alga *Enteromorpha*
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, *Corophium* sp., ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	15-25°C
4. Salinity:	Variable, species dependent
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 5 - 8 mm amphipods, 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:	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

REFERENCES:

Adapted from:
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, *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 MAYFLY, *Hexagenia limbata*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	17°C, 20-22°C
4. Salinity:	freshwater
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 800 mL
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	young nymphs
13. No. organisms per test chamber:	5 minimum
14. No. replicate chambers per concentrations:	4 minimum
15. No. organisms per concentration:	1-10
16. Feeding regime:	Variable
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:	None
22. Endpoint:	Survival
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability:	≥ 80% survival in controls

* - Protocol Dependent

REFERENCES:

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

Bedard, D., A. Hayton and D. Persaud. 1992. Ontario Ministry of the Environment laboratory sediment biological testing protocol. Ontario Ministry of the Environment, Toronto, Ontario. 26 pp.

* 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 THE POLYCHAETE, *Neanthes arenaceodentata,* ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	20 ±1°C
4. Salinity:	20-35 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	12L/12D
8. Test chamber size:	1 L
9. Test solution volume:	Vol. to 800 mL
10. Sediment depth:	2.5 cm (200 mL)
11. Renewal of test solutions:	None*
12. Age of test organisms:	2-3 weeks
13. No. organisms per test chamber:	5 maximum
14. No. replicate chambers per concentration:	3-5
15. No. organisms per concentration:	15-25
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 eqivalent 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

REFERENCES:

ASTM. 1994. Method E1611-94. Standard guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Dillon, T.M., D.W. Moore and A.B. Gibson. 1993. Development of a chronic sublethal bioassay for evaluating contaminated sediment with the marine polychaete worm, *Nereis (Neanthes) arenaceodentata.* Environ. Toxicol. Chem. 12:589-605.

Reish, D.J. 1992. Guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. ASTM Draft No. 5. July 3, 1992. 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 PAPER PONDSHELL FRESHWATER MUSSEL, *Anodonta imbecillis*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	24±1°C
4. Salinity:	0 ‰
5. Light quality:	N/A
6. Light intensity:	N/A
7. Photoperiod:	24 h Dark
8. Test chamber size:	5 cm-diam. glass cylinder closed on lower end with 100 µm Nitex, placed in 250 mL glass dish containing test sediment and overlying water
9. Test solution volume:	150 mL overlying water
10. Sediment depth:	0.5 cm (20 mL)
11. Renewal of test solutions:	None*
12. Age of test organisms:	8-10 d post transformation to juveniles
13. No. organisms per test chamber:	10
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	50 minimum
16. Feeding regime:	Daily; bloomed phytoplankton concentrate @6 mL/L
17. Test chamber cleaning:	None
18. Test solution aeration:	None
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 filtered non-toxic natural freshwater
20. Test concentrations:	Site sediment, a reference sediment and a control sediment
21. Dilution series:	N/A
22. Endpoint:	Survival (death assumed if absence of ciliary action or empty shells)
23. Sampling and sample holding requirements:	<8 wk
24. Sample volume required:	2 L
25. Test acceptability criterion:	≥ 80% survival in controls

REFERENCES:

Keller, A.K., and S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Andonata imbecilis*. Environ. Toxicol. Chem. 10:539-546.

Warren, L.W. and S.J. Klaine. 1995. The development of freshwater mussel bioassays to characterize sediment toxicity. N. Am. Benthol. Soc. (In Press).

Tennessee Valley Authority Draft Standard Operating Procedures, SOP-21, and personal communication from Don Wade, Tennessee Valley Authority.

* 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/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. 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 COMMERCIAL SHRIMP, *Penaeus* sp., ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	25±1°C
4. Salinity:	30-35 ‰ ±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:	80 L minimum
9. Test solution volume:	60 L 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:	8-10 d post larvae
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:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	If needed to maintain DO> 40% saturation (< 100 bubbles/min.)
19. Dilution water:	Natural seawater or modified GP2, Forty Fathoms® or equivalent, artifical 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:	20 L for site sediment and 8 L for reference and control sediment
25. Test acceptability criterion:	≥ 80% survival in controls

REFERENCE:

Modified from the mysid acute toxicity water column test published in:

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

* 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 GRASS SHRIMP, *Palaemonetes* sp., ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	25±1°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:	16L/8D
8. Test chamber size:	80 L minimum
9. Test solution volume:	60 L 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:	1-4 d from hatch
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:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	If needed to maintain DO > 40% saturation (< 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:	20 L for site sediment and 8 L for reference and control sediment
25. Test acceptability criterion:	≥ 80% survival in controls

REFERENCE:

Modified from the mysid acute toxicity water column test published in:

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

* 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 NAIDID OLIGOCHAETE, *Pristina leidyi*, ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 d
3. Temperature:	24±1°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:	250 mL
9. Test solution volume:	10 g (wet wt)/50 mL overlying water
10. Sediment depth:	2 cm minimum
11. Renewal of test solutions:	None*
12. Age of test organisms:	Mixed age
13. No. of organisms per test chamber:	5
14. No. replicate chambers per concentration:	5
15. No. organisms per concentration:	25
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	None
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:	500 mL
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

Smith, D.P., J.H. Kennedy and K.L. Dickson. 1991. An evaluation of a naidid oligochaete as a toxicity test organism. Environ. Toxicol. Chem. 10: 1459-1465.

* 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 OLIGOCHAETE, *Tubifex tubifex,* ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 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:	250 mL
9. Test solution volume:	100 mL
10. Sediment depth:	100 mL
11 Renewal of test solutions:	None*
12. Age of test organisms:	Mixed age
13. No. organisms per test chamber:	5
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	25
16. Feeding regime:	None
17. Test chamber cleaning:	None
18. Test solution aeration:	None
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:	1 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

Adapted from:

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.

Reynoldson, T.B., S.P. Thompson and J.L. Bamsey. 1991. A sediment bioassay using the tubified oligochaete worm *Tubifex tubifex*. Environ. Toxicol. Chem. 10:1061-1072.

* 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 OLIGOCHAETE, *Lumbriculus variegatus,* ACUTE TOXICITY SEDIMENT TESTS

1. Test type:	Static Non-renewal*
2. Test duration:	10 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:	300 mL minimum
9. Test solution volume:	100 mL minimum
10. Sediment depth:	3 cm
11 Renewal of test solutions:	None*
12. Age of test organisms:	Mixed age
13. No. organisms per test chamber:	10
14. No. replicate chambers per sediment:	5
15. No. organisms per sediment:	50
16. Feeding regime:	10 mg trout chow starter on days 0, 5
17. Test chamber cleaning:	None
18. Test solution aeration:	None
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:	1 L
25. Test acceptability criterion:	≥ 90% survival in controls

REFERENCES:

Adapted from:

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.

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.

Bailey, N.C. and D.N.W. Lui. 1980. *Lumbriculus variegatus*, a benthic oligochaete, as a bioassay organism. Pp. 202-215. In: J.C. Eaton, P.R. Parrish and A.C. Hendricks (Eds). *Aquatic Toxicology.* ASTM STP 707. 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.

Sediment Bioaccumulation Tests

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE POLYCHAETE, *Neanthes arenaceodentata,* SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Static Renewal
2. Test duration:	28 d
3. Temperature:	20±1°C
4. Salinity:	20-35 ‰
5. Light quality:	Ambient Laboratory
6. Light intensity:	10-20 uE/m^2/s (50-100 ft-c)
7. Photoperiod:	12L/12D
8. Test chamber size:	1 L minimum
9. Test solution volume:	200 mL overlying water
10. Sediment depth:	2.5 cm (200 mL)
11. Renewal of test solutions:	Weekly
12. Age of test organisms:	2-3 wk
13. No. organisms per test chamber:	5 maximum
14. No. replicate chambers per concentration:	5 minimum
15. No. organisms per concentration:	25 minimum
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 eqivalent 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:

ASTM. 1994. Method E1611-94. Standard guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. Annual Book of ASTM Standards, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA.

Dillon, T.M., D.W. Moore and A.B. Gibson. 1993. Development of a chronic sublethal bioassay for evaluating contaminated sediment with the marine polychaete worm, *Nereis (Neanthes) arenaceodentata.* Environ. Toxicol. Chem. 12:589-605.

Reish, D.J. 1992. Guide for conducting sediment toxicity tests with marine and estuarine polychaetous annelids. ASTM Draft No. 5. July 3, 1992. American Society for Testing and Materials, Philadelphia, PA.

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 POLYCHAETE, *Arenicola marina,* SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Flow-through or Static Renewal
2. Test duration:	28 d
3. Temperature:	10 to 20°C
4. Salinity:	≥ 25‰
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:	1-2 L
9. Test solution volume:	> 500 mL/beaker (e.g., four 1 L beakers in 8 L aquarium)
10. Sediment depth:	≥ 15 cm deep sediment (wet wt); minimum 400 g sediment (wet wt) plus 3.5 g sediment per g wet flesh weight per day (≤ 250 mm in grain size diameter)
11. Renewal of test solutions:	Flow-through = 5-10 vol/d; Static Renewal = 3x/week
12. Age of test organisms:	< 1 year (3-6 g wet weight, 5-10 cm length), larger organisms require more sediment, larger test chambers
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 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:	1 L per treatment, minimum
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. U.S. Environmental Protection Agency. 232 pp.

Gordon, D.C., J. Dale and P.D. Keiger. 1978. Importance of sediment-working by the deposit-feeding polychaete *Arenicola marine* on the weathering rate of sediment-bound oil. J. Fish Res. Bd. Canada. 35:591-603.

Huttel, M. 1990. Influence of the lugworm *Arenicola marina* on porewater nutrient profiles of sand flat sediments. Mar. Biol. Prog. Ser. 62:241-248.

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.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE CLAM, *Yoldia limatula,* SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Flow-Through or Static Renewal
2. Test duration:	28 d
3. Temperature:	5 - 20°C (activity minimal at lowest temperature)
4. Salinity:	≥28‰
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:	500 - 1000 mL (beaker)
9. Test solution volume:	>750 mL/beaker
10. Sediment depth:	100 - 300 g sediment (dry wt), depth greater than shell length. *Yoldia* actively resuspends sediments into water column, additional sediment may need to be added during test to maintain minimal sediment depth.
11 Renewal of test solutions:	Flow-through = 5-10 vol/d; Static Renewal = 3x/week
12. Age of test organisms:	1 - 2 cm g
13. No. organisms per test chamber:	One (1) per beaker
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(s), 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:	1 L, minimum
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. (ATS Deliverable).

Bender, K. and W.R. Davis. 1984. Effects of feeding on *Yoldia limatula* on bioturbation. Ophelia 23: 91-100.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR THE AMPHIPOD, *Diporeia* sp., SEDIMENT BIOACCUMULATION TESTS

1. Test type:	Static Non-renewal* or Overlying Water Renewal
2. Test duration:	28 d
3. Temperature:	4°C
4. Salinity:	0-20 ‰
5. Light quality:	Red darkroom light
6. Light intensity:	Low
7. Photoperiod:	Continuous
8. Test chamber size:	4 L minimum
9. Test solution volume:	to 4 L
10. Sediment depth:	3 cm
11 Renewal of test solutions:	Variable
12. Age of test organisms:	Mixed age juveniles
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 (>10g OC/g organism)
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 water; synthetic water modified to reflect receiving water hardness or salinity to 20%
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:

Landrum, P.F. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod, *Pontoporeia hoyi*. Environ. Sci. Technol. 23:588-595.

Landrum, P.F., B.J. Eadie and W.R. Faust. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* spp. Environ. Toxicol. Chem. 10:35-46.

* 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).