| Test Species, Test Duration, | | | | | Protocol |
|---|---|--|--|--|---|
| and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Reference |
| Amphipod Ampelisca abdita 10 Day Acute Sediment Test 28 Day Chronic Sediment Test | 10 Day Survival, Reburial. 28 Day Growth, Reproduction | Highly reliable test; laboratory exposure analogous to field conditions; very useful in defining gradients of sediment toxicity; readily available and widely distributed species; test salinities from 10 to 35 ppt; tolerant of fine sediments; high regulatory relevance; mortality endpoint has high ecological relevance; moderately cost- effective | Tube dweller, not in direct contact with sediment; sensitive to coarse- grained sediments; species is field-collected | Less sensitive than <i>E. estuarius</i> and <i>R abronius;</i> LC50 value for un-ionized ammonia is 0.83 mg/L (Kohn et al., 1994) | Test Method 100.4 (EPA 1994); ASTM E1367-99 EPA 1998 |
| Amphipod <i>Eohaustorius</i> <i>estuarius</i> 10 Days Sediment | Survival, Reburial | Highly reliable test; laboratory exposure analogous to field conditions; very useful in defining gradients of sediment toxicity; readily available species; test salinities from 2 to 28 ppt; tolerant of fine sediments; directly exposed to sediments; high regulatory relevance; mortality endpoint has high ecological relevance; moderately cost-effective | Less sensitive than <i>R. abronius; p</i> otential sediment interferences; not as well distributed as <i>L. plummulosus</i> or <i>A. abdita; s</i> pecies is field collected | Remove potential predators from sediment before testing; less sensitive to sulfide than <i>R. abronius</i> (LC50 = 104 μ M total sulfides/L [Knezovich et al., 1995]); <i>E. estuarius</i> mortality is as sensitive as <i>R. abronius</i> mortality; <i>R. abronius</i> nonreburial is more sensitive than <i>E. estuarius</i> nonreburial; <i>E. estuarius</i> mortality more sensitive than <i>N. areanaceodentata</i> biomass, which is more sensitive than <i>N. areanaceodentata</i> mortality; mortality endpoint more sensitive than reburial; LC50 value for total ammonia is 125.5 mg/L and un-ionized ammonia is 2.49 mg/L (Kohn et al., 1994) | Test Method 100.4 (EPA 1994); ASTM E1367-99 EPA 1998 |

Estuarine and Marine Aquatic Bioassays for Use in Sediment Investigations

Estuarine and Marine Aquatic Bioassays for Use in Sediment Investigations (page 2 of 5)

| Test Species, Test Duration, | | | | | Protocol |
|--|--------------------------------------|--|---|--|---|
| and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Reference |
| Amphipod Leptocheirus plumulosus 10 Days Sediment | Survival, Growth, Reproduction | Species is cultured; salinity range of 1.5 to 32 ppt (pore water salinities of less than 1 to 35 ppt [Niewolny et al., 1997]); tolerates full range of grain sizes (except sandy sediments with less than 5% silt/clay [Niewolny et al., 1997]); tolerant of fine sediments; highly reliable test; high ecological relevance; laboratory exposure analogous to field conditions; widely distributed and cultured | Tube dweller – not in direct contact with sediment | | Test Method EPA 600/R-01/020 (2001) |
| Amphipod Leptocheirus plumulosus 10 Days Sediment | Survival, Reburial | As above. | Tube dweller – not in direct contact with sediment | | Test Method 100.4 (EPA 1994); ASTM E1367-99 |
| Amphipod <i>Rhepoxynius</i> <i>abronius</i> 10 Days Sediment | Survival, Reburial | Highly reliable test; most sensitive of amphipods usually tested; laboratory exposure analogous to field conditions; very useful in defining gradients of contamination; readily available species; test salinities from 25 to 32 ppt; directly exposed to sediment; high regulatory relevance; mortality endpoint has high ecological relevance; moderately cost- effective | Sensitive to high total organic content; sensitive to fine grained sediments; not as well distributed as <i>Leptocheirus</i> <i>plummulosus</i> and <i>Ampelisca abdita</i> ; species is field-collected | <i>R. abronius</i> mortality as sensitive as <i>E.estuarius</i> mortality; <i>R. abronius</i> nonreburial is more sensitive than <i>E. estuarius</i> nonreburial; LC50 value for un-ionized ammonia is 1.59 mg/L (Kohn et al., 1994); more sensitive to sulfides than <i>E. estuarius</i> (LC50 for total sulfides is 50 µM total sulfides/L [Knezovich et al., 1995]); 10-day survival protocol using <i>R. abronius</i> was a more sensitive indicator of toxicity than 20-day test with <i>N. arenaceodentata</i> based on statistical power of the test and not greater sensitivity of the organisms or endpoints (Anderson et al., 1998) | Test Method 100.4 (EPA 1994); ASTM E1367-99 EPA 1998 |

| Test Species, Test Duration | | | | | Protocol |
|--|------------------------------------|---|---|---|--|
| and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Reference |
| Diatom Thalassiosira pseudomona Skeletonema costatum Dunaliella tertiolecta Phaeodactylum tricornutum 4 Days Water | Growth, Biomass, Cell Counts | Represents aquatic primary producers; Can be used with filtered porewater | Primarily a water test; not relevant to sediment | Not recommended unless there is clear evidence that Navy activities are currently impacting the water body. | ASTM E 1218-97a |
| Mollusc <i>Macoma balthica</i> 28 Days Sediment | Bioaccumulation | Species is wild-harvested | Ecological relevance; available year-round, surface deposit-feeder; tolerates salinity down to 10 ppt | | ASTM 1668-00 EPA 1998 |
| Mollusc <i>Macoma nasuta</i> 28 Days Sediment | Bioaccumulation | Species is wild-harvested | Ecological relevance; available year-round, common test species; tolerates salinity down to 10 ppt | | ASTM 1668-00 EPA 1998 |
| Mollusc Yoldia imatula 28 Days Sediment | Bioaccumulation | Species is wild-harvested | Ecological relevance; available year-round, subsurface deposit- feeder | Seawater must be >25 ppt | ASTM 1668-00 EPA 1998 |
| Mollusc Blue mussel <i>Mytilus edulis</i> 2 Days Water column | Development | Species is cultured; high dose responsiveness | Moderate ecological relevance; does not spawn year-round | Less sensitive than Strongylocentrotus purpuratus to sulfides (complete inhibition at 8- µM total sulfide/L [Knezovich et al., 1995]) | Test Method 1005.0 (Chapman, 1995) |
| Polychaete <i>Capetella</i> sp. 20-28 Days Sediment | Bioaccumulation | Species can be cultured | Low sensitivity; mortality has moderate dose responsiveness | | ASTM 1668-00 |

Estuarine and Marine Aquatic Bioassays for Use in Sediment Investigations (page 3 of 5)

Estuarine and Marine Aquatic Bioassays for Use in Sediment Investigations (page 4 of 5)

| Test Species, Test Duration, and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Protocol Reference |
|--|---------------------------------|---|---|---|--|
| Polychaete Nereis (Neanthes) areneceo- dentata 20 Days Sediment | Growth Survival | Species is cultured; species can be tested at salinities less than 20 ppt; growth test is cost- effective; low cost; mortality has high dose response; mortality has high ecological relevance | Low to medium sensitivity; mortality has moderate dose responsiveness; growth has moderate ecological relevance | Test animal age, duration of exposure, food ration, and choice of endpoint affects the magnitude of the toxic response (Bridges and Farrar, 1997; Bridges et al., 1997); <i>N. arenaceodentata</i> biomass is more sensitive than <i>N. arenaceodentata</i> mortality | (EPA 1990) ASTM 1611-99 |
| Polychaete <i>Nereis virens</i> 28 Days Sediment | Bioacumulation | Species is cultured, or wild harvest, can tolerate salinities as low as 10 ppt | Cannot be tested with other test species (predator). | Surface deposit-feeder and omnivore. Good provider of biomass. | Test Method 1005.0; (Chapman et al., 1995); ASTM 1688-00 |
| Possum mysid Americamysis bahia 7 Days Water column | Fecundity Survival Growth | Species is cultured | Very sensitive to changes in ionic balance in test solution | Laboratory success with the fecundity endpoint is rare (WSDE, 1997) | EPA Test Method 1007.0 |
| Purple sea urchin <i>Strongylo-</i> <i>centrotus</i> <i>purpuratus</i> 72 Hours Water column | Development Survival | Highly sensitive; early-life stage toxicity test; can be performed using filtered porewater | Species is field-collected; not recommended for sediments with a porewater salinity less than 10 ppt; does not spawn year-round; does not directly live in sediments; moderate ecological relevance | Highly susceptible to unionized ammonia toxicity when testing sediment pore water (EC50 for un-ionized ammonia was 0.057 mg/L [Greenstein et al., 1996]); total inhibition from sulfides at 20-µM total sulfide/L [Knezovich et al., 1995]) | Test Method 1008.0 (Chapman, 1995) |
| Sand dollar Dendraster excentricus 72 Hours Water column | Development Survival | Highly sensitive; early-life stage toxicity test; gravid adults can be obtained year-round (U.S. EPA 1993b); can be performed using filtered porewater | Species is field collected; medium dose responsiveness; moderately cost-effective; moderate ecological relevance | Can be induced to spawn but with reduced gamete viability; proposed echinoderm effect threshold for unionized ammonia is 0.04 mg/L (U.S. EPA, 1993b) | Test Method 1008.0 (Chapman, 1995) |

| Test Species, Test Duration, and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Protocol Reference |
|---|-----------------|--|---------------|---|---------------------------------------|
| Topsmelt Atherinops affinis 12 Days Sediment-water interface | Percent hatched | Most appropriate for testing early life stage fishes; can tolerate salinities from 2 to 60 ppt; can tolerate wide range in temperature with a preference of 19 to 23°C; reproductive season from early March to July or August depending upon latitude | | Suggested test instead of using sediment pore water where fish are receptors of concern; numerically abundant in California estuaries | (Protocol based on Anderson, 1996) |
| Inland silverside <i>Menidia beryllina</i> 7 Days Water column | Survival | Species is cultured; occurs along both coasts of the United States; can tolerate freshwater to salinities of 35 ppt; can tolerate temperatures from 9.8 to 30°C; sexually mature from March or April through July or August | | | EPA Test Method 1006.0 |

Estuarine and Marine Aquatic Bioassays for Use in Sediment Investigations (page 5 of 5)

ASTM =American Society for Testing and Materials.EC50 =Concentration that causes an effect in 50% of the test organisms.LC50 =Concentration that is lethal to 50% of the test organisms.WSDE =Washington State Department of Ecology.

| Test Species | Test Duration and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Protocol Reference |
|--|-----------------------------------|--------------------------------------|--|--|---|---|
| Algae, Selenastrum capricornutum, Scenedesmus subspicatus, Chlorella vulgarius | 4 Days; water | Growth, Biomass, Cell Counts | Represents aquatic primary producers | Primarily a water test. Can be used with filtered pore water extracts | | ASTM E 1218-97a |
| African Clawed Frog (FETAX) <i>Xenopus laevis</i> | 96 Hours; sediment | Development Teratogenesis | Time and cost-effective; technical ease in conducting test; versatile for testing various media; sensitive to low levels of developmental toxicants; extensive literature available; most predictive when compared to lettuce germination, earthworm survival, <i>Daphnia</i> survival, and fathead minnow survival (Fort et al. 1995) | | Can be used to test complex industrial mixtures; has been used to test surface water and aqueous extracts of soil; reference toxicant is 6-aminonicotinamide | ASTM E1439-98 |
| Amphipod <i>Diporeia</i> sp. | 10 Days; sediment | Bioaccumulation | Species can be cultured; pollution tolerant; important component in freshwater food chain | Small size requires massive numbers of individuals for chemical analysis | Subsurface deposit- feeder | ASTM 1688-00 EPA 1998 |
| Amphipod <i>Hyalella azteca</i> | 10 Days; sediment | Survival, Growth | Species is cultured; most highly sensitive of the freshwater test organisms; tolerates wide range of sediment grain sizes | Alkalinity commonly encountered in sediment porewater is toxic (Lasier et al., 1997; Duh and Myers, 1997) | Tolerates salinities up to 15 ppt | Test Method 100.1 (EPA 2000); ASTM E-1706-95b |
| Amphipod <i>Hyalella azteca</i> | 42 Days; sediment | Survival, Growth, Reproduction | Species is cultured; most highly sensitive of the freshwater test organisms; tolerates wide range of sediment grain sizes | Alkalinity commonly encountered in sediment porewater is toxic (Lasier et al., 1997; Duh and Myers, 1997) | Can be used to evaluate the bioavailability of sediment associated contaminants; tolerates salinities up to 15 ppt | EPA/600/R-99/064, ASTM E-1706-95b |

Freshwater Bioassays for Use in Sediment Investigations

| Test Species | Test Duration and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Protocol Reference |
|--|--|---|---|---|--|---|
| Daphnid Ceriodaphnia dubia | 8 Days or until 60% of survivors have three broods; water | Survival, Reproduction | Species is cultured; important link in many food chains; species is as sensitive as fathead minnow; wide hardness tolerance; found throughout the United States | Test was originally developed to support water testing. Sediment elutriates containing massive amounts of fine material can mechanically induce mortality and /or create difficulty observing the very small offspring. | Applicable to testing effluents, leachates, liquid phases of sediments with minimal quantities of "permanently" suspended material, and pore-water | Test Method 1002.0 |
| Fathead Minnow Pimephales promelas, | 7 Days; water column | Survival, Growth | Species is cultured; occurs throughout the United States; eggs and early larvae commercially available; large effects data base | Not typically used in sediment suspended particulate testing | Applicable to testing effluents, leachates, and liquid phases of sediments with minimal quantities of "permanently" suspended material, and porewater | EPA/600/4-91/002 |
| Mayfly - Burrowing, <i>Hexagenia</i> sp. | Life Stage Dependent; sediment | Survival, Growth, Bioaccumulation | Species is cultured; sensitive freshwater test organism; tolerates wide range of sediment grain sizes | Can mature into flying form if test duration extended | Important food chain item for freshwater fish | ASTM 1688-00 |
| Midge Larvae, Chironomus tentans | 10 Days; sediment | Survival, Growth | Species is cultured; sensitive freshwater test organism; tolerates wide range of sediment grain sizes | Can mature into flying form if test duration extended | Important food chain item for freshwater fish | ASTM 1706-95b; EPA Test Method #0100.2 |
| Midge Larvae, Chironomus tentans | 14 Days; sediment | Bioaccumulation | Species is cultured; sensitive freshwater test organism; tolerates wide range of sediment grain sizes | Can mature into flying form if test duration extended, requires many individuals to provide sufficient biomass | Important food chain item for freshwater fish | ASTM 1688-00 EPA 1998; Lifecycle Test Method #0100.5 |

Freshwater Bioassays for Use in Sediment Investigations (page 2 of 3)

Freshwater Bioassays for Use in Sediment Investigations (page 3 of 3)

| Test Species | Test Duration and Medium | Endpoints | Advantages | Disadvantages | Other Remarks | Protocol Reference |
|--|-----------------------------------|-----------------|---|--|---------------|---|
| Oligochaete Lumbriculus variegatus | 28 Days; sediment | Bioaccumulation | Species is easy to culture, know chemical exposure history, tolerant to wide range of sediment types, low acute sensitivity to wide range of chemicals, tolerates long exposures without feeding | Individuals are small and often difficult to remove from sediment and detritus matrix | | ASTM E1688-00, EPA/600/R-99/064 (2000[2]) |

ASTM = American Society for Testing and Materials.

EC50 = Concentration that causes an effect in 50% of the test organisms.

LC50 = Concentration that is lethal to 50% of the test organisms.

WSDE = Washington State Department of Ecology.

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