

Shaded Text reflects January 2013 second edition changes.

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.

Y= Yes, meets requirements; N= No, does not meet requirements; NA= not applicable.

DO = dissolved oxygen; temp = temperature; conc = concentration(s); 3x week = three times weekly; d = day; SD = standard deviation; # = number (of)

TEST SPECIFIC CHECKLIST							
Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
<b>Sample Collection and Handling: Field-Collected Sediments</b>							
Sample Collection	Minimum of five replicate samples (field replicates, i.e. separate samples from different grabs or cores) be taken at each sampling station, including 1 or more reference stations (must)						
	Properties of reference sediment are similar to test sediments						
	A benthic grab or core rather than a dredge be used; sediment be collected from 1 or more depths, ideally capturing the top 2 cm of surficial sediment						
	pH, redox potential and temp be measured in the field to help characterize the sample						
	Care taken to minimize loss of fines during sample collection (must)						
Containers	Made of nontoxic material; new or thoroughly cleaned and rinsed with test water or other clean water (eg: deionized water) before use (must)						
	Each sample container to be filled completely to exclude air						
Volumes	At least 1 L of sediment per sample (field replicate) is normally required						
Labelling	Immediately after filling, each sample container be sealed and labeled or coded (must)						
	Label and/or records include a code or sample identifier, source, location, replicate number, date of collection (must); label includes name and signature of sampler(s)						
Holding Time	Test to be initiated within 6 weeks after sampling (must); recommend within 2 weeks after sampling						
	Date of receipt of the sample(s) at lab to be recorded (must)						
Holding Conditions	Sample temp measured and recorded upon receipt at lab						
	Upon collection, warm (>7 °C) samples be cooled to between 1 and 7 °C with regular ice or frozen gel packs, and kept cool (4 ± 3 °C) in darkness throughout transport						
	Samples be kept from freezing (or partially freezing) or drying out during transport or storage (must)						
	Samples and subsamples held in airtight containers, in darkness at 4 ± 2 °C (must); with no headspace						

### TEST SPECIFIC CHECKLIST

#### Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca*

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Subsample Mixing	Each subsample to be thoroughly remixed to ensure homogeneity before use <b>(must)</b>						
Sample Handling	Has sediment sample handling guidance in EPS 1/RM/29 been cited in lab's SOP?						
<b>Sample Preparation : Field-Collected Sediments</b>							
Filtering and Sieving	Normally remove debris and indigenous macro-organisms using forceps; filtering and sieving only if necessary						
Homogenization	Homogenize sample and subsample (including any separated liquid) before use						
	Mixing conditions (type, duration, temp) as similar as possible for each sample <b>(must)</b> Immediately following sample mixing, subsamples be removed and placed in labelled test vessels or containers <b>(must)</b>						
Characterization	For whole sediment, particle size analysis (% coarse- med- fine- sand, silt and clay) and TOC for each replicate sample <b>(must)</b> ; measurement of pore water and/or whole sediment pH and ammonia, and percent water content is recommended						
Description	Qualitative description of each sample when the test is being set up including sample colour, texture, homogeneity, presence of plants, animals, tracks or burrowing animals						
Pre-aeration	Overlying water aerated overnight before test organisms are added						
<b>Sample Preparation: Spiked Sediment</b>							
Solution preparation	Chemical/sediment mixture prepared by making up a stock solution of the chemical and then remixing one or more measured volumes into control sediment, ensuring homogeneity of chemicals in sediment						
	Chemical(s) to be tested be at least reagent grade						
	Chemical containers be sealed and coded upon receipt in the laboratory <b>(must)</b>						
Solvent	Test water is the preferred solvent for preparing stock solutions						
	If an organic solvent is used, the test is conducted using both a clean sediment control (ie, no solvent and no test substance) and a sediment control containing solvent <b>(must)</b>						
	A solvent control sediment is prepared containing the conc of solubilizing agent that is present in the highest conc of the test chemical in sediment <b>(must)</b>						
	Solvent from the same batch used to make the stock solution is to be used <b>(must)</b>						
	The maximum conc of solvent in the sediment is to be at conc that does not affect the survival and growth of <i>H. azteca</i> during the test Spiking process includes a step which allows the solvent to evaporate before addition of sediment and water						
Spiking and mixing	Wet-spiking recommended over dry-spiking; temp during mixing is to be kept low						
	Mixing conditions are to be standardized for each treatment <b>(must)</b> Options for mixing: by hand, sediment rolling technique (e.g., using a mixing device),						

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Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Spiking and mixing cont.	sediment suspension technique, slurry spiking technique						
Storage and Equilibration	Period of equilibration after spiking (must), duration is dependant on nature of chemical and sediment						
	Once prepared, each treatment placed in sealed container with no air space and stored in the dark at 4 ± 2 °C for 4 weeks (i.e., equilibrated) before use in test						
Pre-aeration	Overlying water aerated overnight before test organisms are added						
<b>Sample Collection and Handling: Water-Only Testing</b>							
Containers	Collapsible polyethylene/polypropylene containers used for transporting drinking water						
Holding Conditions	Upon collection, warm (>7 °C) samples be cooled to between 1 and 7 °C with regular ice or frozen gel packs, and kept cool (4 ± 3 °C) in darkness throughout transport						
	Samples be kept from freezing (or partially freezing) during transport or storage (must) Upon arrival at the laboratory, temp of the sample is measured and recorded (must)						
Subsampling for Water Renewal	Two procedures are acceptable: (i) single sample collection, divided into three subsamples (must) (ii) if toxicity is known/anticipated to change substantially, fresh samples are collected (or, in the case of elutriate, prepared) on at least three separate occasions using sampling intervals of 4-6 days or less (must)						
	First subsample (or fresh sample) used for test initiation (Day 0) plus the first two renewals, the second subsample (or fresh sample) for the 3rd and 4th renewals, and the third subsample (or fresh sample) for the 5th and 6th renewals (must)						
Holding Time	Test to be commenced within 3 days of sample collection or elutriate preparation (must)						
	Samples of sediment collected for the purposes of elutriate extraction and testing are tested within 10 day of collection (must)						
Sample Volume	60 to 80 L						
<b>Sample Preparation: Water-Only Testing</b>							
Field-collected	Water sample or subsample in a collection container be agitated thoroughly before pouring (must)						
	Filtration normally not recommended; if sample is filtered, use 60-µm sieve (must)						
	Subsamples are mixed together (must)						
Chemical Testing	Test solutions typically prepared by adding aliquots of a stock solution						
	If stock solutions are used, conc and stability of test chemical in solution is determined						
	Unstable stock solutions are prepared three times weekly, or more frequently if necessary (must)						

### TEST SPECIFIC CHECKLIST

#### Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca*

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Chemical Testing cont.	If solvent (or equivalent) is used, an additional control solution be prepared with the conc of solubilizing agent that is present in the most concentrated solution of the test chemical (must)						
Preparation of solutions	Control solution(s) be prepared at the same time as the experimental treatments (must)						
	Any dilution water used to prepare test concentrations be used for preparing one set of controls (must)						
	Each test solution be mixed well using a glass rod, Teflon™ stir bar, or other device made of nontoxic material (must)						
DO and pH	Measured before use (must)						
Temp	Adjusted to 23 ± 2 °C before use (must)						
Pre-aeration	No pre-aeration unless a test solution has DO<40% or >100% saturation upon preparation, in which case aerate all test solutions for ≤ 20 minutes (or 40% or 100% DO) at minimal rate before starting test or renewing solution						
pH adjustment	No adjustment if pH of test solutions is in range 6.0 to 8.0						
<b>Test Conditions</b>							
Facility and Apparatus	All apparatus and supplies are nontoxic (must)						
	Able to maintain daily mean temp required for sediment and water (must)						
	Have the basic instruments to monitor water quality (test water and pore water) (must)						
	All test vessels, equipment, and supplies that might contact sediment or test water, be clean and rinsed with test water, deionized water or distilled water before use (must)						
	Compressed air used for aerating water is free of oil and fumes (must)						
	Ventilation system prevents cross-contamination from sample storage, sample testing and culturing facilities; ventilation system prevents exposure of personnel to harmful fumes						
Test Type	Whole sediment toxicity test: static (must)						
	If overlying water is fouled/deteriorates, test is conducted as (or shifted to) a static-renewal exposure with 3x week renewal, if test objectives warrant this (must)						
Test Type cont.	Trigger values for static-renewal: ammonia (> 0.2 mg/L unionized NH <sub>3</sub> -N mg/L), pH (<6.0 or >8.0), and/or DO (<40%) of test water						
	Water-only test: static-renewal						
Duration	14 d						
Temperature	Daily ave: 23 ± 1 °C, instantaneous: 23 ± 3 °C (must)						
Lighting	Overhead full spectrum (fluorescent or equivalent); 500 - 1000 lux						
Photoperiod	16 h light: 8 h dark						

TEST SPECIFIC CHECKLIST							
Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
DO range	40% and 100% saturation						
Aeration	Sediment tests (static and static-renewal): continuous and minimal (2 to 3 bubbles) in each test chamber, and <b>does not disturb the sediment (must)</b> ; checked daily						
	<b>Water-only: none, unless DO in overlying water &lt; 40% saturation and renewals cannot ameliorate</b>						
Vessel Size, Type And Identification	<b>Glass beakers or jars (must)</b> ; 300 mL high form beaker or jar; $\geq$ 7 cm inner diameter; normally <b>covered</b>						
	Each test chamber be clearly coded or labeled to enable identification of the sample or its conc <b>(must)</b>						
	The date and time when the test is started to be recorded <b>(must)</b>						
Randomization	Treatments should be positioned randomly within the test facility						
Test volume (sediment:water)	100 mL sediment : 175 mL water <b>(recommended)</b>						
	<b>100 mL sediment : 400 mL test water (or alternate 1:4 ratio which maintains a minimum of 55 mL of sediment)</b>						
	<b>Water-only: 275 mL test solution</b>						
Water-Only Substrate	<b>Substrate added to each vessel (must)</b> ; identical for each vessel <b>(must)</b> ; options for substrate: pre-soaked gauze, Nitex® or plastic mesh, or thin layer of clean silica sand						
Renewal of Solution	Static test: none, except for replacement for losses due to evaporation						
	Static-renewal test option <b>allowed under specific conditions: overlying water is renewed 3x week (minimum, on non-consecutive days), at a rate of two volume additions in 24 hours (must)</b> ; replacement of water performed manually or with automated water-renewal apparatus						
	<b>Water-only test</b>	<b>3x week (minimum, on non-consecutive days), at a rate of &gt; 80% solution renewal (must)</b>					
		<b>any uneaten food and other detritus on the bottom of each vessel should be removed</b>					
		<b>renewal performed cautiously (must) to prevent any injury or accidental loss of any amphipods</b>					
	<b>Siphoned or displaced solution should be saved and checked for amphipods</b>						
Test Water	Culture water or other clean ground or surface water; site water; water adjusted to hardness of site water; reconstituted freshwater for higher degree of standardization; natural or reconstituted seawater with salinity $\leq$ 15 g/kg for test with estuarine sediment; DO 90 - 100 % saturation						

TEST SPECIFIC CHECKLIST							
Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Water cont.	Have been demonstrated to allow acceptable survival and growth of test organisms in 14 d tests with control sediment before use in test <b>(must)</b>						
	Adjusted to 23 ± 1 °C before use <b>(must)</b>						
	When site water is used as overlying water or control/dilution water, a second set of controls is prepared using appropriate laboratory water <b>(must)</b>						
	Water-only: If receiving or "upstream" water used as the control/dilution water, it should be filtered through ≤60-µm sieve						
Control Sediment	Sample of clean sediment that is used to assess the performance of the test organisms and the acceptability of the test <b>(must)</b> ; either natural or formulated sediment can be used						
	Each sediment toxicity test includes an experimental control, with a minimum of 5 replicate vessels per control sediment <b>(must)</b>						
Age of organisms	2- to 9-days old, and ranging in age by ≤ 3 days are used to start test <b>(must)</b>						
# Organisms/ Vessel	10 amphipods added to each test vessel <b>(must)</b>						
	Amphipods to be placed below the air/water interface in overlying water <b>(must)</b> ; assigned randomly						
# Test conc. (chemical testing)	For a multi-conc test, at least 7 conc. plus a control are to be prepared <b>(must)</b> ; more are recommended						
# Replicates/Conc.	Field-collected sediment: ≥ 5 replicate samples (field replicates) per sampling station and per reference site/control sediment <b>(must)</b> ; ≥ 5 replicate vessels (laboratory replicates) for control sediment <b>(must)</b>						
	Spiked sediment: ≥ 5 replicate vessels (laboratory replicates) per treatment <b>(must)</b>						
	Water-only: ≥5 test vessels per conc/treatment <b>(must)</b>						
Feeding Regime	Amphipods are given one of three food options <b>(must)</b> : (1) an aqueous suspension of YCT; (2) finely ground commercial fish food flakes or (3) a 1:1 combination of YCT and finely ground commercial fish food flakes						
	Frequency is daily or 3x week on non-consecutive days <b>(must)</b>						
	Daily feeding: 2.7 mg solids, dry weight (or equivalent) added to each test vessel <b>(must)</b>						
	3x week feeding: 6.3 mg dry solids (or equivalent) added to each test vessel <b>(must)</b>						
	No feeding on Day 14						
Vessel Cleaning	Each beaker be cleaned thoroughly before and after use and rinsed well with test water immediately before use <b>(must)</b>						
Spiked sediment (chemical testing)	If solvent used: results for the two controls are compared to each other using Student's t-test <b>(must)</b>						

TEST SPECIFIC CHECKLIST								
Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>								
Parameter	Specification	Document Review			Implementation			
		Y	N	NA	Y	N	NA	
Biological Endpoints	Survival and final dry weight at end of test							
Statistical Endpoints	Mean (± SD) % of amphipods that survived the 14 d exposure, for each treatment <b>(must)</b>							
	Mean (± SD) dry weight per surviving amphipod, calculated from the total weight of the group of survivors <b>(must)</b>							
	Site comparison	Mortality: logistic regression or alternate tests which address test objectives						
		Growth: ANOVA or equivalent methods which address test objectives						
	Multi-concentration	Mortality: 14d LC50 and 95% confidence limits, calculated using probit or logit regression, Spearman-Kärber or binomial method (based on partial effects) <b>(must)</b>						
		Growth: ICp and 95% confidence limits for dry weight, calculated using regression analysis as the principal technique, provided assumptions are met <b>(must)</b>						
		Growth: initial plot of raw data (% fertilization) against log conc highly recommended; any major disparity between graphic and computer derived ICp resolved <b>(must)</b>						
		Growth: data assessed for outliers <b>(must)</b>						
		Growth: assumptions of normality and homoscedasticity are met <b>(must)</b>						
		Growth: more than one model is attempted and model with best-fit is chosen <b>(must)</b>						
		Growth: endpoints generated by regression analysis are bracketed by test concentrations, i.e. no extrapolation <b>(must)</b>						
		Growth: if data is hormetic, (i) enter directly if regression is used, or (ii) enter control responses entered for those concentrations which demonstrated hormesis if ICPIN is used <b>(must)</b>						
		Growth: if regression analysis is not suitable for data (e.g., assumptions cannot be met), ICPIN is used <b>(must)</b>						
Mortality and weight analyzed separately ("Option 1"); biomass calculation ("Option 3") optional								
If concentrations of chemical were measured (i.e. analyzed), results (including any ICp) are reported using measured concentrations								

TEST SPECIFIC CHECKLIST								
Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>								
Parameter	Specification	Document Review			Implementation			
		Y	N	NA	Y	N	NA	
<b>Observations and Measurements</b>								
Temp <sup>1</sup>	Sediment: start of the test and 3x week (must) Water-only: daily (must)							
DO <sup>1</sup>	Sediment: start of the test and 3x week (must) Water-only: at test start and end, and before and after each test solution renewal (3x week) (must)							
pH <sup>1</sup>	Sediment: for reference sediment, start of the test and 3x week; for all other treatments, start and end of the test (must) Water-only: at test start and end, and before and after each test solution renewal (3x week) (must)							
Ammonia <sup>1</sup>	Sediment: for reference sediment, start of the test and 3x week; for all other treatments, start and end of the test (must) Water-only: at test start and end, and before and after each test solution renewal (3x week) (must)							
Conductivity <sup>1</sup>	Sediment: start and end of test (must) Water-only: at test start and end, and before and after each test solution renewal (3x week) (must)							
Hardness, alkalinity <sup>1</sup>	Sediment: start and end of test Water-only: start and end of test							
Inspection/cleaning of probes	Any probe (DO, pH, conductivity) inserted into a test vessel is inspected (for test organisms) and rinsed with clean water between sample measurements (must)							
Chemical concentration	Spiked sediment: stock solutions, overlying water, sediment, pore water, and test solutions (if studied) be analyzed to determine the chemical conc							
	Water-only: chemical conc measured in aliquots from high, medium, and low test conc and control; if conc declined by > 20%, repeat test with more frequent renewals							
Appearance of test substance	Any change in the appearance of the sediment or overlying water (sediment) or test solutions (water-only)							

<sup>1</sup> For temp, DO, pH, ammonia, conductivity, hardness and alkalinity, measurements are made in the overlying water (sediment) or test solutions (water-only), in at least one test vessel representing each treatment or replicate sample, including control sediment/water. If sediment test is static renewal, water quality measurements should be conducted at the start and end of each renewal period, in both the fresh and the used overlying water just before it is changed, or just after it has been changed.



TEST SPECIFIC CHECKLIST								
Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>								
Parameter	Specification	Document Review			Implementation			
		Y	N	NA	Y	N	NA	
Amphipod behaviour	Regularly check (preferably daily) each test vessel, to observe and record the number of amphipods swimming, floating on the water surface, or lying/grazing on the surface of the sediment							
	Any animals seen floating on the water surface should be gently pushed down into the water using a glass rod or pipette							
Mortality	Water-only: dead and moribund amphipods counted daily							
	At test end, all live animals recovered from the overlying water or sediment in a single test chamber are counted, placed together in a numbered weighing boat and rinsed in test water (must)							
	Missing individuals are assumed dead							
	Inactive individuals prodded gently with a sharp point to confirm mortality							
Growth	Separate weighing boats, each containing the group of surviving amphipods recovered from each test chamber, are dried in an oven for 24 h at 60 °C							
	Upon removal from oven, boats are moved immediately to desiccator (must)							
	The boats be randomly removed from the desiccator and weighed on a balance that measures accurately to 10 µg							
	First boat weighed be replaced in the desiccator and weighed again at end of all weighings; change should not be >5%							
	Mean dry weight per amphipod which survived at test end to be calculated for each group (must)							
Receiving water Used as Control	Water-only: Survival and final dry weight in the laboratory control water must be compared to that in the sample of receiving/upstream water (must)							
Solvent	If both solvent and clean sediment control meet the test validity criteria, the results for the two controls be compared using Student's t-test (must)							
	If the results for the two controls are not statistically different from each other, then only the data from the clean control sediment should be used to calculate the test results							
	If results for two controls are statistically different from each other, further evaluation needed							
<b>Test Organism</b>								
Species	<i>Hyalella azteca</i> (must)							
	Species identification confirmed and documented (must)							
Source	Existing government, private, or commercial culture							
	All amphipods used in a test derived from the same population (must)							

### TEST SPECIFIC CHECKLIST

#### Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca*

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Importation	Transported as young as possible						
	Written statement that identifies the number and source, age, date and time of shipment <b>(must)</b>						
	From a culture that have met health criteria and quality assurance outlined in RM/33 <b>(must)</b>						
	Appropriate culture conditions and water quality requirements be followed by supplier <b>(must)</b>						
	Testing laboratory establish in-house system for health evaluation for each shipment <b>(must)</b>						
	Temp and DO in the water in the shipping container(s) measured and recorded upon departure from the supplier's facility, and on arrival at the testing laboratory <b>(must)</b>						
	During transportation, the temperature water be maintained at or near the required test conditions, not change by more than 3°C during any 24-h period in transit, DO be ≥80% saturation <b>(must)</b>						
	Before shipment, water used for transport well-oxygenated <b>(must)</b>						
	On arrival, organisms are gradually acclimated to holding/testing conditions <b>(must)</b>						
	Holding conditions the same as the test conditions for temperature, light and photoperiod <b>(must)</b>						
	Test organisms that will be used within the first 24 to 48 hours after arrival at the testing facility be cultured by the supplier in water that has similar qualities as the laboratory's water						
	If not used for immediate testing, acclimation be started upon arrival at the testing facility, and be completed 2 days prior to setting up a test						
	For test organisms imported for immediate use in testing, reference toxicant testing is conducted concurrently with 14-d test <b>(must)</b>						
Age	Juvenile <i>H. azteca</i> that have been cultured in a lab and between 2 to 9 days old <b>and range in age by ≤ 3 days</b> at the start of the test <b>(must)</b>						
	Amphipods removed from known age culture as < 1 to 7 d old individuals and held for observation in 750 mL of culture water within 1L beaker for 2 d preceding test; fed daily; <b>density should not exceed 1 amphipod/10mL solution to avoid growth inhibition</b>						
Health Criteria	Discard batch of organisms intended for use in a test if > 20 % of young amphipods die or appear stressed during the 48 h period <b>(24 h if imported)</b> before test <b>(must)</b>						
	Individuals that appear unhealthy (e.g., discoloured, or otherwise stressed), inactive, or dead not be used for testing <b>(must)</b>						

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Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i>								
Parameter	Specification	Document Review			Implementation			
		Y	N	NA	Y	N	NA	
<b>Culture/Holding Conditions</b>								
Facility and Apparatus	Controlled temperature laboratory facility <b>(must)</b>							
	Culturing area isolated from any testing, sample storage or sample preparation areas <b>(must)</b>							
	All equipment, containers and accessories that might contact the organisms or water within the culturing facility are to be clean, rinsed as appropriate, and made of nontoxic materials <b>(must)</b>							
	Toxic materials (copper, zinc, brass, galvanized metal, lead and natural rubber) not come in contact with apparatus and equipment or the culture water <b>(must)</b>							
Water temperature	23 ± 1 °C as daily average and 23 ± 3 °C as instantaneous							
DO and aeration	Aerated gently (1 bubble/s for each liter of water); maintain DO ≥ 80 % saturation							
Lighting	2000 - 2500 lux adjacent to the water surface; overhead full spectrum tubes (fluorescent or equivalent, with a broad spectrum wavelength)							
Photoperiod	16 h light: 8 h dark							
Substrate	presoaked medicinal cotton gauze strips (recommended), Nitex®, nylon mesh, plastic mesh, or shredded paper towels							
Handling	As little as possible; done gently, carefully, quickly to minimize stress; transferred organisms released below the water surface							
	Any animals that are dropped, injured, contact dry surfaces, or appear stressed not to be used for testing <b>(must)</b>							
Age Cultures	Recommend to maintain both mixed age and known age cultures							
Feeding	Commercial fish food flakes, rabbit or trout chow, algae, yeast and/or Cerophyll; various types, quantities and rates allowed							
Water	Untamminated ground, surface, reconstituted, or, if necessary, dechlorinated municipal tap water; reconstituted or natural seawater with salinity ≤ 15 g/kg for special needs							
	If reconstituted fresh water is used for culturing, the five-salt reconstituted water (SAM-5S) is recommended							
	If municipal drinking water is used, dechlorination removes any harmful concentration of residual chlorine or chloramines <b>(must)</b>							
	Temp monitored daily							
	DO monitored at least weekly							
	pH, hardness, alkalinity and ammonia measured during 24 h period preceding start of test							

<b>TEST SPECIFIC CHECKLIST</b>							
<b>Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i></b>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Water Renewal	Intermittent renewal or continuous flow; ≥ 1 volume addition per day recommended; 25 - 30 % per week (minimum) unless water is recirculated through a filtration system						
Monitoring	Checked three times per week (minimum) or daily (preferred)						
Acclimation	Gradually (≤ 2°C /d) for temperature differences upon arrival						
<b>QA/QC</b>							
Validity Criteria	Invalid test if mean 14 d survival in control sediment < 80 % at the end of the test <b>(must)</b>						
	Invalid test if average dry weight for replicate control groups at test end is < 0.1 mg per surviving amphipod <b>(must)</b>						
	Tests using solvent control: if test results in either solvent control or clean control sediment fail to meet validity criteria, test is invalid <b>(must)</b>						
Reference Toxicant	Reagent grade CuSO <sub>4</sub> , CdCl <sub>2</sub> , KCl, or NaCl						
	Frequency is within 14 d of test start or concurrently with definitive or water-only test <b>(must)</b>						
	A static, 96 h water-only reference toxicity test is recommended; may be supplemented or replaced with one or more spiked sediment tests with reference toxicant(s)						
	Substrate added to each test vessel, and is identical for each test vessel in test <b>(must)</b> Options for substrate: presoaked medicinal gauze bandage, Nitex® or plastic mesh, or thin layer of clean silica sand						
	96 h water-only test: uses 2 to 9 d old amphipods that range in age by ≤ 3 days at test start; 10 individuals per test chamber; at least 5 test conc. plus a control (control/dilution water only); 1 or more replicates per treatment; test volume is 200 mL solution per chamber; no aeration; test vessels covered; food equivalent to 0.9 mg dw (three feeding options) is added to each test chamber on Days 0 and 2; daily observations for # of dead or moribund amphipods in each test chamber; DO and temperature measured daily; pH, alkalinity, hardness, and conductivity measured at the start and end of the test; other conditions are similar as those for definitive sediment toxicity test						
	Endpoints are mean % survival in each treatment and 96h LC50 <b>(must)</b>						
	Invalid test if the mean survival in control water is < 90% at test end <b>(must)</b>						
Warning Chart	Prepared for each reference toxicant and continually updated <b>(must)</b>						
	Log conc used in all calculations of mean and standard deviation, and in all plotting procedures <b>(must)</b>						
	Each new LC50 for the reference toxicant be compared with established limits of the chart <b>(must)</b>						
	Acceptable warning limits are ± 2 SD of global mean						

**TEST SPECIFIC CHECKLIST**

**Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca***

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
<b>Test Report (all items here are required, i.e. must)</b>							
Test substance or material	Description of sample type or coding, <b>if and</b> as provided to the lab personnel						
	Information on labeling or coding of each sample						
	Date of sample collection; data and time sample(s) received at lab						
	<b>For wastewater or receiving water samples collected for water-only tests: information on each subsample</b>						
	<b>For effluent or leachate for water-only tests: temp of sample upon receipt at lab</b>						
	<b>For samples or subsamples of wastewater or receiving water collected for water-only tests: pH and DO before use in test</b>						
Test Organism	<b>For samples of elutriate or any liquid extracted from sediments or similar solids, dates for sample generation and use; description and procedure for preparation</b>						
	Species and source of brood stock and test organisms						
	Range of age, at start of test						
	% of young amphipods in known age culture that died or appear to be dead or inactive during the 48h period <b>(24 h if imported)</b> immediately preceding the test						
Test Facilities	Any unusual appearance or treatment of the organisms, before their use in the test						
	Name and address of test laboratory						
	Name of person(s) performing the test						
Control/Dilution Water	<b>Brief description of test vessels (size and shape)</b>						
	Type(s) and source(s) of test water <b>and/or control/dilution water</b>						
	Measured characteristics of test water, before and/or at start of test						
Test Method	<b>Type and quantity of any chemical(s) added to control/dilution water in water-only tests</b>						
	Citation of biological test method used						
	<b>For water-only tests, brief description of procedure(s) in those instances in which a sample, subsample, or test solution has been filtered, or adjusted for pH</b>						
	Design and description if specialized procedure or modification of standard test method						
	Brief description of frequency and type of observations and <b>all</b> measurements made during test						
Test Conditions	Program(s) and methods used for calculating statistical endpoints						
	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in the test method document						
Test Conditions	# of discrete samples per treatment; # of replicate test <b>vessels</b> for each treatment, <b>if applicable</b> ; # and description of treatments in each test including the control(s); test concentrations if applicable						

<b>TEST SPECIFIC CHECKLIST</b>							
<b>Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i></b>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Conditions cont.	Sediment tests: depth and volume of sediment and overlying water in each test chamber Water-only: depth and volume of test solutions, including controls						
	Type of substrate used for water-only tests						
	# of organisms per test vessel and treatment						
	Water-only: brief statement (including procedure, rate, and duration) if any pre-aeration of test solutions						
	Sediment tests: time interval between preparation of test sediment and test initiation (i.e. equilibration time)						
	Test option chosen (static, static renewal for sediments, or water-only); frequency and rate of renewal						
	Food type, feeding regime and ration						
	Indication of any aeration of overlying water (for sediments) or test solutions (for water-only tests); including rate, during exposure of test organisms						
	Dates when test was started and ended						
	For each sediment sample (including each field replicate and all samples of control and reference sediment): particle size distribution (% of coarse-grained sand, medium-grained sand, fine-grained sand, silt, and clay) and total organic carbon content, as well as pore-water and/or whole sediment pH and ammonia (total and un-ionized conc)						
	For sediment tests: all measurements of temp and DO in overlying water for each treatment made at start of test and 3x times per week thereafter, including test end; all measurements of ammonia and pH for each reference sediment made at start of test and 3x times per week thereafter, including test end all measurements of conductivity, pH, and ammonia in overlying water, made at start and end of test for each treatment;						
	For water-only tests: all measurements of temperature (daily), as well as pH, DO, conductivity, and ammonia (at test start and before and after each test solution renewal) in test solutions (including controls), made during the test						
	Date when the reference toxicity test was performed, and any deviations from reference toxicity test procedures						
Test Results	For each replicate (or replicate sample), including each of the control replicates: # and percentage of mortalities, and the dry weight of surviving amphipods at test end						
	For each treatment, including controls -- mean ± SD for % of amphipods that survived the 14d exposure; mean ± SD for dry weight of surviving amphipods at test end; results of any statistical comparisons						

<b>TEST SPECIFIC CHECKLIST</b>							
<b>Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod <i>Hyalella azteca</i></b>							
Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Results cont.	Any LC50 (with 95% confidence limits and if calculated, the slope) determined and indication of quantal method used						
	Any ICp (with 95% confidence limits) determined for the data on growth; details regarding any transformation of data that was required, and indication of quantitative statistic used						
	Type and results of any statistical analysis performed to determine significant differences between field sampling stations (e.g., logistic regression, contrast analysis, contingency tables)						
	Type and results of any model fit or significance of parameters tests from logistic regression (if performed)						
	Any outliers, and justification for their removal or continued inclusion in the data set						
	For a multi-conc test with spiked sediment, indication as to whether results are based on nominal or measured conc of a particular substance or material						
	Results for any 96h LC50 (with 95% confidence limits) performed with the reference toxicant(s) using the same batch of test organisms, together with the geometric mean value (± 2 SD) for the same reference toxicant(s) as derived at the test facility in previous tests using the procedures and conditions herein						
Anything unusual about the test, any problems encountered, any remedial measures taken							
Information Kept On-File	Do lab SOPs indicate that the additional reporting requirements in Section 8.2 of the EPS 1/RM/33 method must be kept on file for 5 years? For details of this information, see EPS 1/RM/33, section 8.2.						

**Notes:**