This checklist is a summary of the requirements and recommendations in the Environment and Climate Change Canada (formerly 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 and Climate Change Canada test method, the test method is taken as the definitive source. Green shaded text reflects changes in the 3rd edition (published September 2017).

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(s); h = hour(s); s = second(s); SD = standard deviation; # = number (of)

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Sample Collection and Handling: Field-Collected Sediments							
Sample Collection	14-d test – minimum of five replicate samples (field replicates, i.e. separate samples from different grabs or cores, stored in separate containers) are taken at each sampling station, including 1 or more reference stations **(must)**: 42-d test – only one 'field replicate' required from each site **(must)**; including 1 or more reference stations	☐	☐	☐	☐	☐	☐
	Properties of reference sediment are similar to test sediments	☐	☐	☐	☐	☐	☐
	A benthic grab or core rather than a dredge is used; sediment is collected from 1 or more depths, ideally capturing the top 2 cm of surficial sediment	☐	☐	☐	☐	☐	☐
	Unstable sediment characteristics (e.g., pH, redox potential, and temp) or those impacted by exposure conditions (e.g., thin sediment layer) are ideally measured in the field to help characterize the sample	☐	☐	☐	☐	☐	☐
	Same sample collection procedure is used at all field sites and stations sampled	☐	☐	☐	☐	☐	☐
	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 is filled completely to exclude air	☐	☐	☐	☐	☐	☐
Volumes	At least 1 L of sediment per sample (field replicate) is normally required; 42-d test – required volumes are confirmed prior to sample collection **(must)**	☐	☐	☐	☐	☐	☐
Labelling	Immediately after filling, each sample container is sealed and labeled or coded **(must)**	☐	☐	☐	☐	☐	☐
	Label and/or records includes code or description identifying sample type, source, location, replicate number, date of collection **(must)**; label includes name and signature of sampler(s)	☐	☐	☐	☐	☐	☐
Holding Time	Test is initiated within 6 weeks (longer only if contaminants are known to remain stable) after sampling **(must)**; recommend within 2 weeks (1 week preferable) after sampling	☐	☐	☐	☐	☐	☐
	Date of receipt of the sample(s) at lab is recorded **(must)**	☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Holding Conditions	Sample temp measured and recorded upon receipt at lab	☐	☐	☐	☐	☐	☐
	Upon collection, warm (> 7 °C) samples are 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 are kept from freezing (or partially freezing) or drying out during transport or storage **(must)**	☐	☐	☐	☐	☐	☐
	Samples and subsamples are held in airtight containers, in darkness at 4 ± 2 °C **(must)**; with no headspace	☐	☐	☐	☐	☐	☐
Subsample Mixing	Each subsample is thoroughly remixed to ensure homogeneity before use **(must)**	☐	☐	☐	☐	☐	☐
Sample Handling	Has sediment sample handling guidance in EPS 1/RM/29 been cited in lab's SOP?	☐	☐	☐	☐	☐	☐
Sample Preparation : Field-Collected Sediments							
Filtering and Sieving	Normally remove debris and indigenous macro-organisms using forceps; filtering and sieving only if necessary	☐	☐	☐	☐	☐	☐
	If sieved with liquid, liquid is remixed with sieved sample **(must)**; If sieved, physicochemical properties are assessed before and after	☐	☐	☐	☐	☐	☐
Homogenization	Homogenize sample and subsample (including any separated liquid) before use	☐	☐	☐	☐	☐	☐
	Mixing conditions (type, duration, temp) are as similar as possible for each sample **(must)**	☐	☐	☐	☐	☐	☐
	Immediately following sample mixing, subsamples are removed and placed in labelled test vessels or containers **(must)**	☐	☐	☐	☐	☐	☐
Characterization	For each sample (including each field replicate and all control and reference samples), particle size distribution (% coarse- med- fine- sand, silt and clay) and TOC for each replicate sample **(must);** measurement of pore water and/or whole sediment pH and ammonia, and percent water content	☐	☐	☐	☐	☐	☐
	Identical chemical, physical, and toxicological analyses are performed with each replicate sample (including reference and control sediment) taken for a study, unless otherwise indicated	☐	☐	☐	☐	☐	☐
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	☐	☐	☐	☐	☐	☐
Sample Preparation: Spiked Sediment							
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) tested are at least reagent grade	☐	☐	☐	☐	☐	☐
	Chemical containers are sealed and coded upon receipt in the laboratory **(must)**	☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
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 **(must)**	☐	☐	☐	☐	☐	☐
	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 **(must)**	☐	☐	☐	☐	☐	☐
	Solvent from the same batch used to make the stock solution is used **(must)**	☐	☐	☐	☐	☐	☐
	The maximum conc of solvent in the sediment is at conc that does not affect the survival, growth and/or reproduction of *H. azteca* 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 kept low	☐	☐	☐	☐	☐	☐
	Mixing conditions are standardized for each treatment **(must)**	☐	☐	☐	☐	☐	☐
	Options for mixing: by hand, sediment rolling technique (e.g., using a mixing device), sediment suspension technique, slurry spiking technique	☐	☐	☐	☐	☐	☐
Storage and Equilibration	Period of equilibration after spiking **(must)**, duration is dependent 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 is aerated overnight before test organisms are added	☐	☐	☐	☐	☐	☐
Sample Collection and Handling: Water-Only Testing							
Containers	Collapsible polyethylene/polypropylene containers used for transporting drinking water	☐	☐	☐	☐	☐	☐
Holding Conditions	Upon collection, warm (> 7 °C) samples are 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 are 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 is commenced within 3 days of sample collection or elutriate preparation **(must)**	☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Holding Time cont.	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	☐	☐	☐	☐	☐	☐
Sample Preparation: Water-Only Testing							
Field-collected	Water sample or subsample in a collection container is 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 3x week, or more frequently if necessary **(must)**	☐	☐	☐	☐	☐	☐
	Solvent used sparingly and at a conc ≤ 0.1 mL/L	☐	☐	☐	☐	☐	☐
	If solvent (or equivalent) is used, an additional control solution is 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) is prepared at the same time as the experimental treatments **(must)**	☐	☐	☐	☐	☐	☐
	Any dilution water used to prepare test concentrations is used for preparing one set of controls **(must)**	☐	☐	☐	☐	☐	☐
	Each test solution is 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	☐	☐	☐	☐	☐	☐
14-d Test Conditions							
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, are 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)**	☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification		Document Review			Implementation		
			Y	N	NA	Y	N	NA
Facility and Apparatus cont.	Ventilation system prevents cross-contamination from sample storage, sample testing and culturing facilities; ventilation system prevents exposure of personnel to harmful fumes		☐	☐	☐	☐	☐	☐
	Testing area isolated from culturing area (**must**)		☐	☐	☐	☐	☐	☐
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**)	☐	☐	☐	☐	☐	☐
		Trigger values for static-renewal: ammonia (> 0.2 mg/L unionized NH_3-N mg/L), pH (< 6.0 or > 8.0), and/or DO (< 40%) of test water overlying reference sediments	☐	☐	☐	☐	☐	☐
	Water-only test: static-renewal (**must**)		☐	☐	☐	☐	☐	☐
Duration	14 d		☐	☐	☐	☐	☐	☐
Temperature	Daily average: 23 ± 1 °C, instantaneous: 23 ± 3 °C (**must**)		☐	☐	☐	☐	☐	☐
Lighting	Overhead full spectrum (fluorescent or equivalent) (**must**); 500 - 1000 lux		☐	☐	☐	☐	☐	☐
Photoperiod	16 h light: 8 h dark (**must**)		☐	☐	☐	☐	☐	☐
DO range	40% and 100% saturation		☐	☐	☐	☐	☐	☐
Aeration	Sediment tests (static and static-renewal): continuous and minimal (2 to 3 bubbles/s) in each test chamber, and does not disturb the sediment (**must**); checked daily		☐	☐	☐	☐	☐	☐
	Water-only: none, unless DO in water < 40% saturation and renewals cannot ameliorate		☐	☐	☐	☐	☐	☐
Vessel Size, Type and Identification	Glass beakers or jars (**must**); 300 mL high-form beaker or jar; ≥ 7 cm inner diameter		☐	☐	☐	☐	☐	☐
	Test vessels covered (covers transparent and allow for some air exchange (**must**), if employed)		☐	☐	☐	☐	☐	☐
	Each test vessel is clearly coded or labeled to enable identification of the sample or its conc (**must**)		☐	☐	☐	☐	☐	☐
	The date and time when the test is started is recorded (**must**)		☐	☐	☐	☐	☐	☐
Randomization	Treatments are positioned randomly within the test facility		☐	☐	☐	☐	☐	☐
Test Volume (sediment:water)	100 mL sediment : 175 mL water		☐	☐	☐	☐	☐	☐
	100 mL sediment : 400 mL test water (or alternate 1:4 ratio which maintains a minimum of 55 mL of sediment)		☐	☐	☐	☐	☐	☐
	Water-only: 275 mL test solution		☐	☐	☐	☐	☐	☐
Water-Only Substrate	Substrate added to each vessel (**must**); identical for each vessel (**must**); options for substrate: pre-soaked gauze, Nitex® or plastic mesh, or thin layer of clean silica sand		☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification		Document Review			Implementation		
			Y	N	NA	Y	N	NA
Renewal of Solution	Static test: none, except for replacement for losses due to evaporation		☐	☐	☐	☐	☐	☐
	Static-renewal test option allowed under specific conditions: overlying water is renewed 3x week (minimum), at a rate of two volume additions in 24 h **(must)**; replacement of water performed manually or with automated water-renewal apparatus		☐	☐	☐	☐	☐	☐
	Water-only test	3x week (minimum), at a rate of > 80% solution renewal **(must)**	☐	☐	☐	☐	☐	☐
		Any uneaten food and other detritus on the bottom of each vessel is removed	☐	☐	☐	☐	☐	☐
		Renewal performed cautiously **(must)** to prevent any injury or accidental loss of any amphipods	☐	☐	☐	☐	☐	☐
		Siphoned or displaced solution is saved and checked for amphipods	☐	☐	☐	☐	☐	☐
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 ≤ 15 g/kg for test with estuarine sediment; DO 90 - 100 % saturation at test temp; pH measured and stable		☐	☐	☐	☐	☐	☐
	Has been demonstrated to allow acceptable survival, and growth of test organisms in tests with control sediment before use in test **(must)**		☐	☐	☐	☐	☐	☐
	Adjusted to 23 ± 1 °C before use **(must)**		☐	☐	☐	☐	☐	☐
	When site water is used as overlying water or control/dilution water, a second set of controls is prepared using appropriate (culture water, unless organisms are imported) laboratory water **(must)**		☐	☐	☐	☐	☐	☐
	Water-only: If receiving or “upstream” water used as the control/dilution water, it is 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 **(must)**; either natural or formulated sediment can be used; shown previously to provide consistent and acceptable endpoints; success with a 14-d test not assumed to demonstrate suitability for a 42-d test **(must)**		☐	☐	☐	☐	☐	☐
	Each sediment toxicity test includes an experimental control, with a minimum of 5 replicate vessels per control sediment **(must)**		☐	☐	☐	☐	☐	☐
Age of Organisms	2- to 9-days old, and ranging in age by ≤ 3 days are used to start test **(must)**		☐	☐	☐	☐	☐	☐
# Organisms/ Vessel	10 amphipods added to each test vessel **(must)**		☐	☐	☐	☐	☐	☐
	Amphipods are placed below the air/water interface in overlying water **(must)**; assigned randomly		☐	☐	☐	☐	☐	☐

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification		Document Review			Implementation		
			Y	N	NA	Y	N	NA
# Test Conc. (Chemical Testing)	For a multi-conc test, at least 7 conc. plus a control are prepared **(must)**; more are recommended		☐	☐	☐	☐	☐	☐
# Replicates/Conc.	Field-collected sediment: ≥ 5 replicate samples (i.e., field replicates with 1 replicate vessel per field replicate) per sampling station and per reference site/control sediment **(must)**; ≥ 5 replicate vessels (i.e., laboratory replicates) for control sediment **(must)**		☐					
	Spiked sediment: ≥ 5 replicate vessels (laboratory replicates) per treatment **(must)**							
	Water-only: ≥ 5 test vessels per conc/treatment **(must)**							
Feeding Regime	Amphipods are given one of three food options **(must)**: (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 **(must)**							
	Daily feeding: 2.7 mg solids, dry weight (or equivalent) added to each test vessel **(must)** 3x week feeding: 6.3 mg dry solids (or equivalent) added to each test vessel **(must)**							
	No feeding on Day 14							
Vessel Cleaning	Each beaker is cleaned thoroughly before and after use and rinsed well with test water immediately before use **(must)**							
Spiked Sediment (chemical testing)	If solvent used: results for the two controls are compared to each other using Student's t-test **(must)**							
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 **(must)**							
	Mean (± SD) dry weight per surviving amphipod, calculated from the total weight of the group of survivors **(must)**							
	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-Karber or binomial method (based on partial effects) **(must)**						
		Growth: ICp and 95% confidence limits for dry weight, calculated using regression analysis as the principal technique, provided assumptions are met **(must)**						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification		Document Review			Implementation		
			Y	N	NA	Y	N	NA
Statistical Endpoints cont.	Multi-concentration cont.	Growth: initial plot of raw data (% fertilization) against log conc highly recommended; any major disparity between graphic and computer derived ICp resolved **(must)**						
		Growth: data assessed for outliers **(must)**						
		Growth: assumptions of normality and homoscedasticity are met **(must)**						
		Growth: more than one model is attempted and model with best-fit is chosen **(must)**						
		Growth: endpoints generated by regression analysis are bracketed by test concentrations, i.e. no extrapolation **(must)**						
		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 **(must)**						
		Growth: if regression analysis is not suitable for data (e.g., assumptions cannot be met), ICPIN is used **(must)**						
		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						
Technician Proficiency	Whole sediment test: Technicians processing vessels have previously demonstrated ≥ 90% recovery of similar-sized amphipods from sediment							
42-d Test Conditions								
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 are 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 & culturing facilities; ventilation system prevents exposure of personnel to harmful fumes							
	Testing area isolated from culturing area (**must)**							
Test Type	Whole sediment toxicity test: static-renewal **(must)**							
Duration	42 d **(must)**							
Temperature	Daily average: 23 ± 2 °C, instantaneous: 23 ± 3 °C **(must)**							

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Lighting	Overhead full spectrum (fluorescent or equivalent) **(must)**; 500 - 1000 lux at surface of overlying water						
Photoperiod	16 h light: 8 h dark **(must)**						
DO range	40% and 100% saturation						
Aeration	Continuous and minimal (2 to 3 bubbles/s) in each test chamber, and does not disturb the sediment **(must)**; checked daily; halted for ~1 h after organisms are added **(must)**						
Vessel Size, Type and Identification	Wide-mouth 1L glass beakers or jars **(must)**						
	Test vessels are covered **(must)**; Covers are transparent **(must)** and contain holes for feeding and aeration						
	Each test vessel is clearly coded or labeled to enable identification of the sample or its conc **(must)**						
	The date and time when the test is started (i.e., organisms added) is recorded **(must)**						
Randomization	Test vessels are positioned randomly within the test facility **(must)**						
Test volume (sediment:water)	18 mL sediment **(must)** : 900 mL water added in a way to minimize disturbance of the sediment and rinse down sediment adhering to sides of vessel **(must)**						
Vessel Preparation	Test vessels with sediment and overlying water are held with aeration at test conditions overnight (or longer for equilibration) before introducing test organisms **(must)**						
Renewal of Solution	Temp adjusted RO or DI replacement of evaporative losses **(must)**						
	~80% of overlying water is renewed at least on days 14, 28, and 35 **(must)**; replacement of water performed manually or with automated water-renewal apparatus; care taken to not disturb sediment or test organisms particularly after young have been produced (i.e., Days 24 to 28); siphon (if used) does not contact or disturb the sediment **(must)**						
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 ≤ 15 g/kg for test with estuarine sediment; DO 90 - 100 % saturation at test temp; pH measured and stable						
	Has been demonstrated to allow acceptable survival, growth, and reproduction of test organisms in tests with control sediment before use in test **(must)**; success with a 14-d test not assumed to demonstrate suitability for a 42-d test **(must)**						
	Water contains ≥ 0.02 mg/L Br and ≥ 15 mg/L Cl (supplemented with NaBr and NaCl if necessary, or if Br conc is not confirmed analytically) **(must)**						
	Adjusted to 23 ± 2 °C before use **(must)**						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Water cont.	When site water is used as overlying water or control/dilution water, a second set of controls is prepared using appropriate laboratory water (culture water, unless organisms are imported) **(must)**						
	When multiple batches of overlying water are prepared all batches are documented with dates prepared & used in addition to the name & amount of any chemicals used **(must)**						
Control Sediment	Sample of clean sediment used to assess the performance of the test organisms and the acceptability of the test **(must)**; either natural or formulated sediment can be used; success with a 14-d test not assumed to demonstrate suitability for a 42-d test **(must)**						
	Has been demonstrated previously to enable validity criteria to be met **(must)**						
	Each sediment toxicity test includes an experimental control, with a minimum of 8 replicate vessels per control sediment **(must)**						
Test Organisms	7- to 9-days old **(must)**, mean dry weight at test start 0.02 – 0.035 mg/individual, measurement at test start strongly recommended for inexperienced labs						
	20 amphipods added to each test vessel **(must)**						
	Amphipods are placed below the air/water interface in overlying water **(must)**; assigned randomly; handled as little and carefully as possible						
	Vessels are examined immediately after organisms are added, and floating or injured organisms are replaced **(must)**						
	30 organisms (i.e., 3 replicates of 10) are collected for initial dry weigh measurements (if required); randomly selected as a subsample of the organisms used for testing **(must)**; rinsed well until free of sediment **(must)**; oven dried for 24-h at 60 ± 5°C, immediately moved to a desiccator, and following cooling, weighed to the nearest 10 µg; first boat weighed is returned to the desiccator and weighed again at end of all other weighing; change is not > 5%						
# Test Conc. (chemical testing)	For a multi-conc test, at least 7 conc. plus a control are prepared **(must)**; more are recommended						
# Replicates/Conc.	Field-collected sediment: ≥ 8 replicate samples (only 1 field replicate per sampling station and per reference site/control sediment) **(must)**; ≥ 8 replicate vessels for control sediment **(must)**; 10 replicates recommended						
	Spiked sediment: ≥ 8 replicate vessels (laboratory replicates) per treatment **(must)**						
Feeding Regime	Food is an aqueous suspension of yeast/cereal grass media/trout chow (YCT) and finely ground commercial fish food flake solution **(must)**; prepared flake solution stored no longer than 5 days; prepared flake solution is refrigerated **(must)**; purchased YCT contains 1.8 g (±0.1) total solids/L **(must)**						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Feeding Regime cont.	Frequency is 3x week **(must)**; no feeding on Day 42 **(must)**						
	Feed amounts (mg solids [dry weight] / per test vessel): Weeks 1 and 2 – 3.15 mg YCT and 3.15 mg fish flakes, Weeks 3 and 4 – 6.3 mg YTC ad 6.3 mg fish flakes, Week 5 to 6 – 12.6 mg YTC and 12.6 mg fish flakes **(must)**						
	Examine vessels for abnormalities (fungal or plant growth, water clarity, organism behaviour, etc) at each feeding **(must)**; DO, pH, and ammonia are measured if water fouling is suspected						
Vessel Cleaning	Each beaker is cleaned thoroughly before and after use and rinsed well with test water immediately before use **(must)**						
Organism Recovery and Biological Observations	Light table is used to provide contrasting background **(must)**						
	Young and adults are recovered (by swirling vessels and decanting portions of overlying water/sediment slurry); young and adults are counted and collected into separate containers; individuals completely inactive but not obviously dead are examined under microscope or hand-held magnifying glass **(must)** prodded with sharp point and observed for 15 s before counted as dead and discarded (not included in weight measurements)						
	Sieving and/or staining not used **(must)**						
	Consistent amount of time is taken to examine each replicate vessel (e.g., 20 to 45 minutes); recovery time for each replicate documented and held on file **(must)**						
	Total # young is recorded **(must)**; total # live and dead adults recovered (unrecovered adults are recorded as dead) is recorded **(must)**						
	Adults are preserved and examined under a dissecting microscope to determine sex **(must)**; technicians are trained and able to distinguish males and females **(must)**						
	Care taken to ensure adult amphipods are free of sediment and debris **(must)**; Groups of surviving adults are dried at 60 ± 5°C for 24 h (see “Growth” in “Observations and Measurements” for additional recommendations)						
Spiked Sediment (chemical testing)	If solvent used: results for the two controls are compared to each other using Student’s t-test **(must)**						
Biological Endpoints	Survival, growth (as biomass), and reproduction **(must)**						
Statistical Endpoints	Mean (± SD) % of adult amphipods that survived the 42 d exposure, for each treatment **(must)**						
	Mean (± SD) dry weight per surviving adult amphipod, calculated from the total weight of the group of survivors **(must)**						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Statistical Endpoints cont.	Mean (± SD) adult biomass **(must)**; adult biomass calculated as total dry weight of surviving adults divided by the initial # organisms **(must)**; adult amphipods accidentally killed, removed, or lost during the test are deducted from initial # organisms for that replicate; a value of zero is assigned if all adults in a particular replicate died during the test						
	Mean (± SD) # young per surviving female per treatment **(must)**; calculated by dividing the total # young produced in a replicate by the # adult female organisms surviving in that replicate at the end of the test **(must)**						
	Mean (± SD) survival normalized reproduction **(must)**; calculated by multiplying the young per surviving female for a replicate by the fraction survival (i.e., males and females) for that replicate **(must)**						
	Statistical analysis of reproduction data is considered in addition to growth and survival data **(must)**; t-test is normally appropriate for comparing test sample to control or reference sediment; a variety of ANOVA and multiple comparison tests (and non-parametric equivalents) are used for comparing multiple sampling stations; guidance in Sections 4.7, 5.6 and 6.5 are consulted for the statistical analysis (i.e., for site comparison and multi-conc tests)						
Technician Proficiency for Organism Identification and Recovery	Staff proficiency is evaluated before conducting a 42-d test for the first time **(must)**						
	1-d exposure using 2-5 day old organisms in 1 L glass test vessels containing 18 mL of wet sediment and 900 mL overlying water **(must)**						
	Variety of sediment types (e.g., with high organic, sand, and/or gravel content) used						
	Minimum of 3 test vessels per technician; maximum of 80 amphipods per test vessel **(must)**						
	Technicians being evaluated are “blind” to the # organisms per vessel **(must)**; Number of organisms per vessel will randomly (e.g., as generated using random number formula in Excel) vary from 20 to 80						
	Recovery attempted at least 2-h after introduction of test organisms on Day 1 but no later than Day 2 **(must)**						
	Procedure used for recovery is the same as that used for the test (Sec. 8.13.1)						
	Recovery time per vessel no longer than 45 minutes **(must)**						
	Results of recovered organisms are compared to actual numbers added **(must)**; minimum average 80% recovery **(must)**, and average 85% recovery recommended						
	Technical proficiency re-evaluated every 3 years unless technician is routinely (i.e., 3 times per calendar year) conducting 42-d tests **(must)**						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Observations and Measurements							
Temp[1]	14-d Sediment: start of the test and ≥ 3x week **(must)**; daily recommended Water-only: daily **(must)** 42-d Sediment: daily **(must)**						
DO[1]	14-d 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)** 42-d Sediment: start and end of the test and ≥ 3x week **(must)**; at each renewal in both old and new water **(must)**						
pH[1]	14-d 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)** 42-d Sediment start and end of the test and ≥ 3x week **(must)**; at each renewal in both old and new water **(must)**						
Ammonia[1]	14-d Sediment: for reference sediment, start of the test and ≥ 3x week; for all other treatments, start and end of the test **(must)**; calculate un-ionized ammonia with concurrent pH measurement Water-only: at test start and end, and before and after each test solution renewal (≥ 3x week) **(must)** 42-d Sediment: start and end of test, and on days 7, 21, and 42 and calculate un-ionized ammonia with concurrent pH measurement **(must)**; total ammonia in old overlying water just before it is changed on days 14, 28, and 35 **(must)**						
Conductivity[1]	14-d 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)** 42-d Sediment: start and end of test, and weekly **(must)**						
Hardness, Alkalinity[1]	14-d and 42-d Sediment: start and end of test Water-only: start and end of test						

[1] 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, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Salinity (if appropriate)	14-d and 42-d Sediment: start and end of test **(must)** Water-only: start and end of test and before and after each test solution renewal (≥ 3x week) **(must)**						
Inspection/ Cleaning of Probes	Any probe (temp, DO, pH, conductivity, salinity) inserted into a test vessel is inspected (for test organisms) and rinsed with distilled, deionized or RO water between sample measurements **(must)**; care taken to not injure test organisms while measuring **(must)**						
Sample Aliquots for Analysis	Aliquots taken from overlying water consumed during analysis (i.e., hardness, alkalinity, ammonia), are taken from extra replicates set up for monitoring or consist of less than 10% of the volume of overlying water (42-d sediment test **(must)**); volume replaced with dilution water						
Chemical Concentration	Spiked sediment: stock solutions, overlying water, sediment, pore water, and test solutions (if studied) are 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)						
Amphipod Behaviour	Regularly check (at each feeding, preferably daily) each test vessel, to observe and record the # amphipods swimming, floating on the water surface, lying/grazing on the surface of the sediment, or in amplexus						
	Any animals seen floating on the water surface are gently pushed down into the water using a glass rod or pipette						
Mortality	Water-only: dead and moribund amphipods are counted daily						
	At test end, all live adults 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 are prodded gently with a sharp point to confirm mortality						
Growth	Separate weighing boats, each containing the group of surviving adult amphipods recovered from each test chamber and rinsed to remove sediment, are dried in an oven for 24 h at 60 ± 5°C						
	Upon removal from oven, boats are moved immediately to desiccator **(must)**						
	The boats are randomly removed from the desiccator and weighed on a balance that measures accurately to 10 µg						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Growth cont.	First boat weighed is returned to the desiccator and weighed again at end of all other weighing; change is not > 5%						
	Mean dry weight per adult amphipod which survived at test end is calculated for each group **(must)**						
Receiving water Used as Control	Water-only: Survival and final dry weight in the laboratory control water are 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 are 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 are used to calculate the test results						
	If results for two controls are statistically different from each other, further evaluation is needed						
Test Organism							
Species	*Hyalella azteca* **(must)**						
	Species identification confirmed and documented **(must)**						
Source	Existing government, private, or commercial culture						
	All amphipods used in a test are derived from the same population **(must)**						
Importation	Transported as young as possible						
	Written statement that identifies the number and source, age, date and time of shipment **(must)**						
	From a culture that have met health criteria & quality assurance outlined in RM/33 **(must)**						
	Appropriate culture conditions & water quality requirements are followed by supplier **(must)**						
	Testing laboratory establish in-house system for health evaluation for each shipment **(must)**						
	Temp and DO in the water in the shipping container(s) are measured and recorded upon departure from the supplier's facility, and on arrival at the testing laboratory **(must)**						
	During transportation water temp is maintained at or near the required test conditions and does not change by more than 3°C during transit						
	DO is ≥ 80% saturation **(must)**						
	Before shipment, water used for transport is well-oxygenated **(must)**						
	On arrival, organisms are gradually acclimated to holding/testing conditions **(must)**						
	Holding conditions are the same as the test conditions for temp, light and photoperiod **(must)**						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Importation cont.	Test organisms that will be used within the first 24 to 48 h after arrival at the testing facility are cultured by the supplier in water that has similar qualities as the laboratory's water						
	If not used for immediate testing, acclimation is started upon arrival at the testing facility, and 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 **(must)**						
	If imported for use in 42-d test, organisms (7 to 9-d old) are acclimated for at least 24 h prior to use **(must)**; < 20% mortality during the 24 h immediately preceding the test **(must)**						
Age	Juvenile *H. azteca* that have been cultured in a lab						
	14-d test – individuals between 2 to 9 days old and range in age by ≤ 3 days at the start of the test **(must)**; 42-d test – individuals between 7 to 9 days old **(must)** with mean dry weight between 0.02 – 0.035 per organism						
	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; density should not exceed 1 amphipod/10mL solution to avoid growth inhibition						
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 (24 h if imported) before test **(must)**						
	Individuals that appear unhealthy (e.g., discoloured, or otherwise stressed), inactive, or dead are not used for testing **(must)**						
Culture/Holding Conditions							
Facility and Apparatus	Controlled temperature laboratory facility **(must)**						
	Culturing area isolated from test, sample storage or sample preparation areas **(must)**						
	All equipment, containers and accessories that might contact the organisms or water within the culturing facility are clean, rinsed as appropriate, and made of nontoxic materials **(must)**						
	Toxic materials (copper, zinc, brass, galvanized metal, lead and natural rubber) does not come in contact with apparatus and equipment or the culture water **(must)**						
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						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Substrate	Presoaked medicinal cotton gauze strips (recommended), Nitex®, nylon mesh, plastic mesh, silica sand, 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 are not used for testing **(must)**						
Age Cultures	Lab maintains both mixed age and known age cultures						
Feeding	Commercial fish food flakes, rabbit or trout chow, algae, diatoms, yeast and/or cereal grass media (e.g. Cerophyll); various types, quantities and rates allowed						
Water	Uncontaminated 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 **(must)**						
	Temp monitored daily						
	DO monitored at least weekly						
	pH, hardness, alkalinity and ammonia measured during 24 h period preceding start of test						
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 3x week (minimum) or daily (preferred)						
Acclimation	Gradually (≤ 2°C /d) for temperature differences upon arrival						
QA/QC							
Validity Criteria	14-d - Invalid test if mean 14-d survival in control < 80 % at the end of the test **(must)**; 42-d – Invalid test if mean 42 d adult survival in control sediment < 80% at the end of the test **(must)**						
	14-d - Invalid test if average dry weight for replicate control groups at test end is < 0.1 mg per surviving amphipod **(must)**; 42-d - Invalid test if average dry weight for replicate control groups at test end is < 0.5 mg per surviving adult amphipod **(must)**						
	42-d – Invalid test if < 6.0 young produced per surviving female **(must)**						
	Tests using solvent control: if test results in either solvent control or clean control sediment fail to meet validity criteria, test is invalid **(must)**						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Reference Toxicant	Reagent grade $CuSO_4$, $CdCl_2$, KCl, or NaCl						
	Frequency is within 14 d of test start or concurrently with definitive or water-only test **(must)**						
	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)						
	Alternative to a multi-concentration ref tox: Positive control with known response is included with each test; % inhibition or stimulation is calculated for each endpoint; response is defined, and acceptability limits (operationally defined) and variability limits are included for each endpoint **(must)**; lab monitors consistency, precision and trends over time; outliers trigger investigations into potential causes **(must)**						
	Substrate is added to each test vessel, and is identical for each test vessel in the test **(must)** 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 dry weight (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; temp measured daily for each treatment; DO, pH, and conductivity measured at the start of the test for each treatment **(must)**; Alkalinity and hardness measured for each treatment at test start and end; other conditions are similar as those for definitive sediment toxicity test						
	Endpoints are mean % survival in each treatment and 96h LC50 **(must)**						
	Invalid test if the mean survival in control water is < 90% at test end **(must)**						
Warning Chart	Prepared for each reference toxicant and continually updated **(must)**						
	Log conc used in all calculations of mean and standard deviation, and in all plotting procedures **(must)**						
	Each new LC50 for the reference toxicant is compared with established limits of the chart **(must)**						
	Acceptable warning limits are ± 2 SD of geometric mean						

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Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Report (all items here are required, i.e. must)							
Test Substance or Material	Description of sample type, if and 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						
	For wastewater or receiving water samples collected for water-only tests: information on each subsample						
	For effluent or leachate for water-only tests: temp of sample upon receipt at lab						
	For samples or subsamples of wastewater or receiving water collected for water-only tests: pH and DO before preparation and use in test						
	For samples of elutriate or any liquid extracted from sediments or similar solids, dates for sample generation and use; description and procedure for preparation						
Test Organism	Species and source of brood stock and test organisms						
	Range of age, at start of test						
	42-d test – starting weight range (dry weight) at test initiation (if needed)						
	% of young amphipods in known age culture that died or appear to be dead or inactive during the 48h period (24 h if imported) immediately preceding the test						
	42-d test – if imported, acclimation period and % mortality during 24-h period immediately preceding test						
	Any unusual appearance or treatment of the organisms, before their use in the test						
Test Facilities	Name and address of test laboratory						
	Name of person(s) performing the test						
	Brief description of test vessels (size and shape)						
Control/Dilution Water	Type(s) and source(s) of test water and/or control/dilution water						
	Measured characteristics of test water, before and/or at start of test						
	Type and quantity of any chemical(s) added to control/dilution water in water-only tests						
	42-d test - identification if water was augmented with Br and Cl or analytical confirmation of minimum Br and Cl concs in overlying water						
Test Method	Citation of biological test method used						
	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						
	Design and description if specialized procedure or modification of standard test method						
	Brief description of frequency & type of observations & all measurements made during test						
	Name and citation of program(s) and methods used for calculating statistical endpoints						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Conditions	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in the test method document						
	# of discrete samples per treatment; # of replicate test vessels for each treatment, if applicable; # and description of treatments in each test including the control(s); test concentrations if applicable						
	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, rate, and ration						
	Indication of any aeration of overlying water (for sediment tests) or test solutions (for water-only tests); including rate, prior to and 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						
	For 14-d sediment tests: all measurements of temp and DO in overlying water for each treatment made at start of test and ≥ 3x week thereafter, including test end; all measurements of ammonia and pH for each reference sediment made at start of test and ≥ 3x 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 temp (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						
	For 42-d test: all measurements in all treatments of temp (daily), conductivity (and salinity if appropriate) (weekly), DO (as mg/L and % saturation) and pH (3x per week), hardness and alkalinity (if measured) at test start and end, and total (with calculated un-ionized) ammonia on Days 0, 7, 14, 28, 35, and 42						

TEST SPECIFIC CHECKLIST

Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Conditions cont.	For 42-d test: measurements of pH, total ammonia, and DO at the beginning and end of each renewal period in both old and fresh overlying water on Days 14, 28, and 35						
	Date when the reference toxicity test was performed, and any deviations from reference toxicity test procedures						
Test Results	14-d sediment and water-only tests: for each replicate (or replicate sample), including each of the control replicates: # and % of mortalities, and the dry weight of surviving amphipods at test end						
	14-d sediment and water-only tests: for each treatment, including controls: mean ± SD for % of amphipods that survived the 14-d exposure; mean ± SD for dry weight of surviving amphipods at test end; results of any statistical comparisons						
	42-d tests: # surviving male and female adults per replicate; mean ± SD % surviving adults (and % CV); replicate and treatment mean ± SD final dry weight (and biomass) of surviving adults (and % CV); # young per replicate (and % CV); treatment mean ± SD survival-normalized reproduction; for each replicate, # young per surviving female; and for each treatment, mean ± SD # young per surviving female (and % CV); and						
	Optional endpoints for 42-d tests: replicate and treatment mean ± SD weight increase (and % CV) if initial weights were measured						
	Any LC50 (with 95% confidence limits) determined and indication of quantal method used for multi-conc tests						
	Any ICp (with 95% confidence limits) determined for the data on dry weight at test end; details regarding any transformation of data that was required, and indication of quantitative statistic used for multi-conc tests						
	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						

TEST SPECIFIC CHECKLIST **Test for Survival, Growth and Reproduction in Sediment and Water Using the Freshwater Amphipod *Hyalella azteca* – 3rd Edition**							
Parameter	**Specification**	**Document Review**			**Implementation**		
		Y	**N**	**NA**	**Y**	**N**	**NA**
Test Results cont.	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 9.2 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, 3rd edition, Section 9.2.						

Notes: