

This checklist is a summary of the requirements and recommendations in the Environment Canada (now Environment and Climate Change 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.
 temp = temperature; conc = concentration(s); d = day; SD = standard deviation; # = number (of); DO = dissolved oxygen;
 CV = coefficient of variation

TEST SPECIFIC CHECKLIST								
Survival and Growth in Sediment Using Polychaete Worms (<i>Polydora cornuta</i>)								
Parameter	Specification	Document Review			Implementation			
		Y	N	NA	Y	N	NA	
Sample Collection and Handling: Field-Collected sediment								
Sample Collection	RM/29 consulted for guidance with respect to sampling design, sampling procedures, and subsample compositing							
	Sediment collected via benthic grab or core from depths that represent the layer(s) of concern							
	Care taken to minimize the loss of fines							
Containers	Made of nontoxic material (Must); new containers or thoroughly cleaned used containers (Must), rinsed before use with test water							
	Filled to exclude air							
	Sealed and labeled/coded (Must)							
Labeling/coding	Include at least sample type, source, precise location, replicate number, date and time of collection (Must); name and signature of sample collectors							
Holding Time	Test to be initiated no later than 6 weeks after sampling (Must)							
	Recommend test initiation within 2 weeks after sampling, preferably within 1 week							
Holding Conditions	Upon collection, if sample > 7 °C, cool to 1 - 7°C with regular ice or frozen gel packs							
	Samples are kept cool (1 - 7°C) in transit using regular ice or frozen gel packs							
	Samples to be kept from freezing or drying during transport and storage (Must)							
	Date of receipt at laboratory recorded (Must); temperature upon receipt recorded							
	Sample held in darkness in airtight containers at 4 ± 2°C (Must); headspace purged with nitrogen before capping							

Characterization	For whole sediment – particle size distribution, % water content, total organic carbon content (Must)						
	For pore water – salinity, pH, and ammonia (total and un-ionized) (Must)						
Sample Preparation : Field-Collected Test Sediment							
Sieving	Sample is not sieved with water (Must) ; indigenous organisms or large debris are moved with forceps, gloved hand or coarse (e.g., 5 mm) sieve						
Homogenization	Each sample of test sediment mixed thoroughly						
	Moisture separated during transport and storage remixed into sample (Must)						
	Mixing conditions (i.e. duration and temp) are as similar as possible (Must)						
	Subsamples for testing and physicochemical analysis taken immediately after mixing (Must)						
Salinity Adjustment	None to interstitial water of test sediments; adjustments are to be made to the overlaying water if necessary						
Sample Preparation: Spiked Sediment							
Chemical	Containers sealed and coded or labeled upon receipt (Must)						
	Chemical to be tested at least reagent grade (if applicable)						
Mixing	Mixing conditions standardized for each treatment included in a test (Must)						
	Mixtures of spiked sediment aged for 4 weeks before testing in sealed containers (no headspace) at $4 \pm 2^\circ\text{C}$ in the dark						
Test Conditions							
Test Facility and Apparatus	Facility is properly ventilated and free from physical disturbances, isolated from culture area and sample preparation/storage area						
	All construction materials that might contact the organisms, water, or test chambers made of nontoxic materials (Must) and materials that minimize sorption						
	Instruments available to measure basic water quality variables (temp DO, pH, salinity, conductivity) for test water and pore water (Must) and lab prepared for other analyses (e.g. ammonia, hydrogen sulphide, residual chlorine)						
	All test chambers, equipment, and supplies that might contact sediment or test water cleaned and rinsed with test water, deionized water, or distilled water. (Must) ; Control/dilution water rinse immediately before use in test						
Test Type	Static with overlying water renewed on Day 7 (Must)						
Test Duration	14 days (Must)						
Test Temp	Daily mean = $23 \pm 1^\circ\text{C}$ (Must) ; instantaneous = $23 \pm 3^\circ\text{C}$ (Must)						

Lighting	Full spectrum (fluorescent or equivalent) (Must) ; 500-1000 lux adjacent to the surface of overlaying water						
	Photoperiod 16 h light, 8 h dark (Must)						
Salinity	Porewater salinity of each test sediment measured (Must) ; salinity between 10-35‰ (Must)						
	Salinity of overlying water within 10-35‰ (Must)						
	Salinity of overlying water within 5‰ of porewater at test start (Must)						
DO	90-100% air saturation						
Aeration	Aeration does not disturb the surface of the sediment (Must) ; overnight before test start and continuously during the test; compressed air free of oil and fumes, oil-free air pumps; 2-3 bubbles/sec; aeration halted temporarily after organism addition to allow the worms to settle						
Test Chambers	300 mL high-form glass beaker with an inner diameter of ~7 cm with watchglass or clear plastic covers						
	Treatments positioned randomly						
	Each vessel clearly labelled or positions coded so that conc. and replicates can be identified (Must)						
	Date and time of test initiation is on labels or data sheets (Must)						
Volume Wet Sediment	50 mL (~2 cm in thickness) (Must)						
Volume Test Water	200 mL (Must)						
Animal Handling	Known-age cultures sieved quickly and gently, worms remaining on sieve transferred rapidly to sorting tray containing test water; worms remaining in mucoid tubes probed to observe vitality; all worms examined under dissecting microscope with atypical (size, colour, injury, etc) being discarded						
	45 worms selected randomly during test setup and divided into 3 groups before being dried to determine initial mean dry weight of the group \pm SD						
Renewal of Test Solution	~80% of overlaying water on Day 7 only (Must) ; water from the same source as test setup (Must) ; water added to each vessel on Day 7 from the same batch (Must) ; care taken during siphoning and addition of renewal water not to disturb sediment surface (e.g., through use of disc)						

Dilution/ Control Water	Uncontaminated natural seawater or reconstituted (artificial) seawater; routinely analyzed including salinity, pH, suspended solids, total organic carbon, pesticides, ammonia, nitrite and metals						
	Artificial seawater prepared by the direct addition of dry salts aerated vigorously for a minimum of 24 h before use (Must)						
	HSB prepared by freezing (-10°C to -20°C for ≥6 h) or evaporation (≤40°C while aerating); salinity 90 ± 1 g/kg						
	HSB must be filtered (≤1 µm) and aerated vigorously for at least 24 h before use (Must)						
	Water previously demonstrated to allow acceptable survival and growth of <i>P. cornuta</i> in 14-d tests w/ negative control sediment, if possible (Must)						
	Water filtered (≤5 µm) no more than 24 h before use; additional filtration (≤0.45 µm) and/or UV sterilization if contamination with pathogens is suspected						
	Adjusted to test temperature before use (Must)						
	Same water used for preparing control and all test conc. (Must)						
	Water added on any day to any test chamber from the same batch (Must)						
	Salinity of batches does not differ by more than 2 ‰						
	pH and salinity measured and stable						
Artificial and natural seawater should be held in clean and covered containers a 4 ± 2°C and used within 14 days							
Initial tests	≥5 control performance tests and ≥5 reference toxicity tests with negative control sediment intended for routine use performed before undertaking toxicity tests						
	Conditions and procedures for initial control performance are identical to those described for the definitive test						
	Conditions and procedures for initial reference toxicity tests are identical to those described for routine reference toxicant tests						
	Data from initial reference toxicity tests are compared by calculating and appraising the magnitude of the coefficient of variation (CV) of the derived LC50s						
Negative Control Sediment	Clean sediment used to assess the performance of the test organisms, the acceptability of the test, and serve as a basis for interpreting data derived from the test sediments; either natural or artificial sediment can be used						
	Negative control sediment is included as a treatment in every toxicity test (Must)						
	During collection, temperature and pore water salinity measured						

Negative Control Sediment (cont)	Sediment composited and analyzed for % water content, particle size distribution, organic content, and concentration of any contaminants						
	Sediment frozen and washed through a fine-mesh (e.g. 0.5 mm) sieve to remove indigenous macro-organisms; sediment allowed to settle overnight, overlying water decanted, and sediment re-mixed; adjustment of porewater salinity permitted						
	Sediment placed in containers sealed to exclude air and stored at -20°C and thawed as required; washed three times after thawing to remove soluble toxic constituents						
Positive Control Sediment	Included in each series of 14-d tests; may be a negative control sediment spiked with a reference toxicant or with one or more toxic chemicals of concern; or a highly contaminated sample of field-collected sediment; or a standard contaminated sediment						
Reference Sediment	One or more samples are included for tests with field-collected sediment, ideally taken from site(s) presumed to be clean but near sites of test soil collection						
	Characteristics such as: percent organic matter, pH, and particle size distribution represent the test sediment as much as possible						
# Replicates/Conc.	≥5 for each treatment or field-sample and control(s) (Must)						
# Animals/Vessel	5 worms per test chamber; randomly transferred						
Feeding Regime	Animals fed three times per week (non-consecutive days) (Must) ; equal ration added to each test chamber on each feeding occasion (Must) ; ration sufficient to enable growth and survival but not excessive (~10 mg dry equivalent per test chamber) (Must)						
	Food type is 1:1 mixtures of ground tropical fishfood flakes and ground <i>Enteromorpha sp.</i> ; prepared and stored on a dry weight basis; a slurry is created to deliver to test vessels						
Chemicals testing: Solvent	Preferred solvent for preparing test solutions is test water; solvent other than water avoided unless absolutely necessary; surfactants not used						
	If organic solvent used – a solvent control (using solvent from the same batch used to prepare the stock solution) included (Must)						
	For tests that included solvent controls – solvent control results statistically compared to negative control data (Must) ; if different than only solvent controls used for comparison to test data; if same both controls should be combined before use in calculating test results or assessing test validity						
Chemicals Testing: # Conc	For a multi-concentration test, ≥5 plus a control (Must) ; 6-10 plus one or more control(s) recommended						

Test Takedown	Worms pipetted from surface of each test chamber to dish for observation; chambers then sieved (shaken in test water – not sprayed) to collect remaining worms; individuals showing no signs of life or potentially remaining in tubes prodded before being counted as dead and discarded; surviving worms dried and weighed; missing individuals counted as dead						
Endpoints	Mean (\pm SD) % of organisms that survived the 14-d exposure (Must)						
	Mean (\pm SD) dry weight per surviving worm, calculated from the total weight of the group of survivors (Must)						
Additional Endpoints (Chemicals Testing)	14 d LC50 with 95% confidence limits (Must) ; 14 d ICp with 95% confidence limits (Must) ; hypothesis testing (e.g., no-observed-effect-concentration) optional						
Observations and Measurements							
Temperature	Measured in one representative test vessel per treatment at beginning of test and thereafter ≥ 3 times per week on non-consecutive days (Must) ; daily recommended						
DO, Salinity, pH, Ammonia	Measured in one representative test vessel per treatment at beginning of test, just before water renewal (Day 7), and at the end of the test (Must) ; any probe used rinsed with distilled or deionized water between samples (Must)						
	More frequent (e.g., 3 x week or daily) DO measurements for sediments with high oxygen demand; DO measured anytime airflow is observed to have stopped (Must) ; any observations of DO dropping below 60% saturation included in test-specific report (Must)						
≥ 3 times per week	Detailed records of food type and ration from each feeding occasion						
	Appearance of the sediment and overlying water in each test chamber						
	Any abnormal events (emerged worms, airflow interrupted, etc.)						
Chemical Concentration (Chemical Testing)	Concentration of chemical in stock solution(s), overlying water, sediment, pore water and test solution (if applicable) is analytically measured						
Test Organisms							
Species	Lab-cultured <i>Polydora cornuta</i> (Must)						
	Species identification confirmed and documented						
Source	Existing government, private, or commercial culture						
	All worms used in a test derived from the same population (Must)						
Age	Juveniles that are 3-4 weeks post-release						
	Mean dry weight of the juveniles used to start the test range within 0.06-0.50 mg per individual (Must)						

Health Criteria	Cultures checked at least 2-3 times per week - emerged adult worms prodded and discarded if they appear to be dead or unhealthy						
	Culture container discarded if ≥20% of adults dead or inactive during any period of observation (Must)						
Culture/ Holding Conditions							
Facility & Apparatus	Controlled temperature laboratory facility with ability to maintain temperature within the required limits (Must)						
	Culturing area isolated from any testing, sample storage, or sample preparation areas						
	All equipment, containers, and accessories that might contact the organisms or water within the culturing facility made of nontoxic materials (Must) ; copper, zinc, brass galvanized metal, lead and natural rubber are not used (Must)						
Temp	23 ± 1°C as daily average (Must) , 23 ± 3°C as instantaneous (Must)						
DO and aeration	Water aerated vigorously just before use; DO 80-100%						
	Culture chambers aerated 2-3 bubbles per second for each L of water; oil-free compressed air delivered through disposable glass pipette, disposable plastic pipette, or aquarium-supply airstone; DO 80-100% saturation						
Salinity	15- 35 ‰ (Must) ; recommended 26-34 ‰						
Lighting	Overhead broad-spectrum; photoperiod 16-h light: 8-h dark; 500 – 1000 lux near the water surface						
Feeding	Diet varies by lifestage: <i>Dunaliella tertiolecta</i> for planktonic larvae stage, <i>D. tertiolecta</i> with increasing quantities of <i>Enteromorpha sp.</i> and tropical fish food flakes for settled juveniles, 1:1 mixture of <i>Enteromorpha sp.</i> and TetraMarin fish food flakes for adults						
	Feeding frequency is 2-3 x per week						
Culture Water	Reconstituted or clean natural seawater						
	Temperature, salinity, DO, and pH measured at least weekly as well as during the 24h period preceding a test.						
	Water quality characteristics (e.g. nitrite, ammonia, hydrogen sulphide, suspended solids, total organic carbon, total dissolved gases, metals, pesticides) analyzed routinely						
Culture Substrate	Field-collected sediment sieved (0.5 mm) then washed with culture water before being frozen; thawed sediment washed three times and used quickly						
	Laboratory formulated negative control sediment can be used as a culture substrate instead of field-collected sediment						

Monitoring	Cultures observed frequently (> 2-3 times per week); documentation of: # of larvae used to begin culture, survival and weight of larvae as they develop, # of surviving adults, young production, dates of culture renewals, feeding regime, etc.						
Water Renewal	Renewed routinely (80-100% weekly water exchange) or continuous recirculation through commercial filter						
Acclimation	Gradual acclimation for temperature ($\leq 2^{\circ}\text{C} / \text{d}$) and salinity ($\leq 5\text{‰} / \text{d}$) differences upon arrival						
QA/QC							
Test Validity Criteria	Test is invalid if the mean percent survival rate in negative control sediment is <90% (Must)						
Reference Toxicant Test	96-h water-only reference toxicant test using cadmium chloride						
	Stock solution made day of use						
	Concentrations of stock solutions and low, medium and high test concentrations are measured chemically						
	If stored, aliquots are acidified and held in the dark at $4 \pm 2^{\circ}\text{C}$ (Must)						
	Performed monthly or concurrently with definitive test (Must) ; performed on new cultures before their use in testing						
	Same test chambers as sediment test; 200 mL of test water per chamber						
	10 individuals per test chamber; aged 3-4 weeks at test start						
	≥ 5 test concentrations plus a control; ≥ 1 replicate per treatment						
	Control/dilution water is culture water; salinity $28 \pm 2 \text{‰}$						
	No aeration or feeding						
	Temp and lighting conditions same as sediment test						
	Observations: daily for dead individuals; test start and end for temp, DO pH, and salinity						
	Endpoint is mean % survival; 96-h LC50						
Test is invalid if the mean survival rate in control water is <90% (Must)							
Warning Chart	Prepared using 96-h LC50 results for each reference toxicant and continually updated (Must)						
	LC50 is acceptable if within warning limits (± 2 SD on log scale)						
	If LC50 outside warning limits (mean ± 2 SD), a thorough check of all procedures and culturing/test conditions is carried out						
	Co-efficient of variation should be <30% and ideally is <20%						

Test Report							
Sample Data	Brief description of sample type as provided to the lab (Must)						
	Information on labelling or coding, for each sample (Must)						
	Date of sample collection; date and time sample received at lab (Must)						
Test Organism	Species and source of brood stock and test organisms (Must)						
	Range of age at start of test (Must)						
	Dry weight (mean ± SD) at start of test (Must)						
	Any unusual appearance, behaviour, or treatment of the organisms before their use in the test (Must)						
Test Facilities	Name and address of test laboratory (Must)						
	Name of person(s) performing the test (Must)						
Test Water	Type and source and salinity of test water (Must)						
	Measured characteristics of test water before and/or at test start (Must)						
Test Method	Citation of biological test method used (Must)						
	Design and description if specialized procedure used or modification of the standard Biological Test Method (Must)						
	Brief description of frequency and type of all observations and all measurements made during test (Must)						
	Program(s) and methods used for calculating statistical endpoints (Must)						
Test Conditions	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in test method document (Must)						
	# of discrete samples per treatment, # of replicates for each treatment, # and description of treatments in each test including controls (Must)						
	Depth and volume of sediment and overlaying water in each test chamber (Must)						
	# of organisms per test chamber and treatment (Must)						
	Feeding regime and ration (Must)						
	Dates when test was started and ended (Must)						
	For each sample, all measurements of sediment - particle size, % water content, and total organic carbon as well as porewater - salinity, pH, and ammonia (Must)						
	For at least one test chamber representing each treatment, all measurements of: temperature, DO, salinity, ammonia, and pH in overlaying water (Must)						

Test Results	For each treatment – mean ± SD for % of worms that survived (Must) ; mean ± SD for dry weight of individual surviving worms (Must) ; results of any statistical comparisons (Must)						
	Co-efficient of variation for mean % survival and mean individual dry weight of replicate control groups (Must)						
	Any LC50 (including 95% confidence limits and slope if determined) (Must)						
	Any ICp (including 95% confidence limits) determined for the data on dry weight (Must) ; details regarding any transformation of data that was required and indication of quantitative statistic used (Must)						
	For a multi-concentration test with chemical spiked sediment, indication if results are based on nominal or measured concentrations as well as all values of measured concentrations (Must)						
	Results of any 96-h LC50 (including 95% confidence limits) performed with a reference toxicant in conjunction with a definitive sediment test with the geometric mean ± 2 SD from previous reference toxicant tests (Must)						
	Anything unusual about the test, any problems encountered, any remedial measures taken (Must)						
Information Kept On-File	Do lab SOPs indicate that the information on Section 7.2 of the EPS 1/RM/41 method must be kept on file for a minimum of 5 years? (Must) For details of this information, see EPS 1/RM/41, Section 7.2						

Notes: