TEST SPECIFIC CHECKLIST

April 1999

Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges *C. tentans* or *C. riparius*

Parameter	Specification	Met Specifics? Y	N	NA
Sample Preparation				
Filtering	Only if necessary, normally remove debris and indigenous macro-organisms using forceps; if necessary, remove smaller macro-organisms using fine-mesh sieve (0.25 to 0.5 mm)	☐	☐	☐
Homogenization	Homogenize sample (including any separated liquid) before use	☐	☐	☐
	For each sample included in a test, mixing conditions (duration, T°) are to be as similar as possible **(Must)**	☐	☐	☐
Characterization	For whole sediment, at least particle size analysis (% sand, silt and clay), TOC, and % water; for porewater, at least pH and ammonia **(Must)**	☐	☐	☐
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	☐	☐	☐
Test Conditions				
Test Facility	All construction materials to be nontoxic **(Must)**	☐	☐	☐
	Able to maintain daily mean T° required for sediment and water **(Must)**	☐	☐	☐
	Have the basic instruments to monitor the quality of the test water and pore water **(Must)**	☐	☐	☐
	All test chambers, 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)**	☐	☐	☐
Test Type/Duration	10d whole sediment toxicity test with 2 options: daily renewal of overlying water (renewal test option) or no renewal (static test option)	☐	☐	☐
Test T°	Daily average to be 23 ± 1 °C and 23 ± 3 °C as instantaneous measurement **(Must)**	☐	☐	☐
Lighting	Overhead full spectrum (fluorescent or equivalent); 500 - 1000 lux; 16 h light: 8 h dark	☐	☐	☐
Aeration	Renewal test option: none, unless D.O. in overlying water drops below 40% saturation	☐	☐	☐
	Static test option: continuous and minimal (2 to 3 bubbles/s) in each test chamber	☐	☐	☐
	Tip of aeration pipette to be above the sediment surface	☐	☐	☐
	Air flow to each test chamber to be gentle and regulated to 2 to 3 bubbles/second **(Must)**; usually checked daily	☐	☐	☐
Test Chamber	300 mL high form glass beaker or glass jar; ~ 7 cm inner diameter; normally uncovered if daily renewal test and covered if static test	☐	☐	☐
Vessel Identification	Each test chamber be clearly coded or labeled to enable identification of the sample or its concentration **(Must)**	☐	☐	☐
	The date and time when the test is started to be recorded **(Must)**	☐	☐	☐
Volume Wet Sediment	100 mL	☐	☐	☐
Volume Test Water	175 mL	☐	☐	☐
Water Renewal	Static test option: none, except for replacement for losses due to evaporation	☐	☐	☐
	Renewal test option: overlying water is replaced at a rate of 2 volumes additions per day usually using an automated water-renewal apparatus	☐	☐	☐
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; D.O. 90 - 100 % saturation	☐	☐	☐
	Have been demonstrated to allow acceptable survival and growth of test organisms in 10d tests with control sediment before use in test **(Must)**	☐	☐	☐
	Adjusted to 23 ± 1 °C before use **(Must)**	☐	☐	☐
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	☐	☐	☐
	Each sediment toxicity test includes an experimental control, with a minimum of 5 replicate beakers per control sediment **(Must)**	☐	☐	☐

TEST SPECIFIC CHECKLIST

April 1999

Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges *C. tentans* or *C. riparius*

Parameter	Specification	Met Specifics? Y	N	NA
Age Confirmation	If *C. tentans* used as test organism, collect a minimum of 20 larvae randomly selected from the culture on Day 0 for head capsule width measurements to confirm their instar **(Must)**	☐	☐	☐
	If *C. riparius* used as test organism, they are to be first instars that are ≤ 48h post-hatch on Day 0 **(Must)**	☐	☐	☐
# Organisms/Vessel	10 organisms to be assigned randomly to each test chamber on Day 0	☐	☐	☐
	Organisms placed below the air/water interface in overlying water **(Must)**	☐	☐	☐
Field Replicates	Recommend ≥ 5 field replicates, each a discrete (different) sample from the same collection site	☐	☐	☐
Lab Replicates	≥ 5 laboratory replicates for each field replicate **(Must)**	☐	☐	☐
Feeding Regime	Ground tropical fish food flakes but 2 options: 1) fed daily with 6.0 mg (dw) added as 1.5 mL inoculum of a 4 mg/L suspension of dry food in water added to each test chamber or 2) fed 4 times only (on non-consecutive days) with 15.0 mg (dw) added as 3.75 mL inoculum of a 4 mg/L suspension of dry food in water added on each feeding to each test chamber **(Must)**	☐	☐	☐
	Identical food ration be added to each test chamber on each feeding occasion **(Must)**	☐	☐	☐
Vessel Cleaning	Each beaker be cleaned thoroughly before and after use and rinsed well with test water immediately before use **(Must)**	☐	☐	☐
Endpoints	Mean (± SD) % of organisms that survived the 10 day exposure **(Must)**	☐	☐	☐
	Mean (± SD) dry weight per surviving organism, calculated from the total weight of the group of survivors **(Must)**	☐	☐	☐
	10d LC50 for multi-concentration test, where appropriate; ICp for weight where appropriate	☐	☐	☐
	Calculation of the mean (± SD) head capsule width for the group of survivors in each treatment is also recommended as a useful endpoint	☐	☐	☐
Observations & Measurements				
Water Renewal	Renewal test option: if automated water renewal apparatus used, system to be monitored daily	☐	☐	☐
D.O. + T°	In overlying water, at the start of the test and ≥ 3 times/week (on non-consecutive days) in at least 1 test chamber per treatment **(Must)**	☐	☐	☐
pH + Conductivity + Ammonia	In overlying water, at start and end of test in at least 1 test chamber representing each treatment **(Must)**	☐	☐	☐
	Probe to be rinsed with clean water between sample measurements **(Must)**	☐	☐	☐
Hardness/Alkalinity	In overlying water, at start and end of test in at least 1 test chamber representing each treatment	☐	☐	☐
Emerged Organisms	# of organisms in each test chamber seen on sediment surface, and their behaviour to be observed daily	☐	☐	☐
Survival	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)**	☐	☐	☐
Growth	Separate weighing boats, each containing the group of surviving organisms 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	☐	☐	☐
	Mean dry weight per organism which survived at test end to be calculated for each group **(Must)**	☐	☐	☐
Test Organisms				
Species	*Chironomus tentans* or *Chironomus riparius* **(Must)**	☐	☐	☐
	Species identification confirmed and documented	☐	☐	☐
Source	Existing government, private, or commercial culture	☐	☐	☐
	All midges used in a test derived from the same population **(Must)**	☐	☐	☐

TEST SPECIFIC CHECKLIST

April 1999

Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges *C. tentans* or *C. riparius*

Parameter	Specification	Met Specifics? Y	N	NA
Age	Third instar *C. tentans* or first instar *C. riparius* at the start of the test **(Must)** . At that time, *C. tentans* has a mean head capsule width of 0.38 mm (range 0.33 to 0.45 mm) and are 9 to 13 days post-hatch and *C. riparius* has a mean head capsule width ~ 0.1 mm (range 0.09 to 0.12 mm) and are < 1 to 3 days post-hatch	☐	☐	☐
Health Criteria	Discard any larvae that appear dead or inactive when gently prodded **(Must)**	☐	☐	☐
Culture/Holding Conditions				
T°	23 ± 1 °C as daily average and 23 ± 3 °C as instantaneous	☐	☐	☐
D.O.	Aerated gently (1 bubble/s for each liter of water); maintain D.O. ≥ 80 % saturation	☐	☐	☐
Lighting	500 - 1000 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	Silica sand or pulp from shredded, unbleached paper towels; recommended depth 1 cm	☐	☐	☐
Handling	As little as possible; done gently, carefully, and quickly to minimize stress	☐	☐	☐
	Any animals that are dropped, injured, contact dry surfaces, or appear stressed not to be used for testing **(Must)**	☐	☐	☐
Feeding	Ground tropical fish food flakes (eg Tetrafin™ or Nutrafin™); various quantities and rates allowed; recommend daily feeding of 300 mg dry weight per 20 L culture chamber containing 150 to 200 *C. tentans* or 250 to 500 *C. riparius*	☐	☐	☐
Water Source	Uncontaminated ground, surface, reconstituted, or, if necessary, dechlorinated municipal tap water; reconstituted or natural seawater with salinity ≤ 15 g/kg for special needs	☐	☐	☐
Water Quality	T° monitored daily	☐	☐	☐
	D.O. monitored at least weekly	☐	☐	☐
	pH, hardness, alkalinity and ammonia measured during 24h 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	☐	☐	☐
Acclimation	Gradually (≤ 2°C /d) for temperature differences upon arrival	☐	☐	☐
Facility & Apparatus	Controlled temperature laboratory facility **(Must)**	☐	☐	☐
	Culturing area isolated from any testing, sample storage or sample preparation areas **(Must)**	☐	☐	☐
	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 **(Must)**	☐	☐	☐
	Toxic materials (copper, zinc, brass, galvanized metal, lead and natural rubber) not come in contact with apparatus and equipment or the culture water **(Must)**	☐	☐	☐
QA/QC				
Test Validity Criteria	Invalid test if mean 10 d survival in control sediment < 70 % at the end of the test **(Must)**	☐	☐	☐
	Invalid test if average dry weight for replicate control groups at test end is < 0.6 mg per surviving organisms if *C. tentans* is used or < 0.5 mg if *C. riparius* is used **(Must)**	☐	☐	☐
Reference Toxicant	Reagent grade copper sulfate, cadmium chloride, potassium chloride, or sodium chloride are recommended	☐	☐	☐
	Reference toxicity test with one or more of these chemicals be performed monthly with the lab's established cultures **(Must)**	☐	☐	☐

TEST SPECIFIC CHECKLIST

April 1999

Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges *C. tentans* or *C. riparius*

Parameter	Specification	Met Specifics? Y	N	NA
Reference Toxicant (con't)	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)	☐	☐	☐
	96 h water only test: uses second instar midge larvae of either species 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; recommended test volume is 200 mL solution per chamber; no aeration; test chambers covered; 1.25 mL of suspension of finely ground commercial fish food flakes comprised of 4 mg of dry solids/mL be added to each test chamber on Days 0 and 2; daily observations for # of dead or moribund organisms in each test chamber; other conditions are similar as those for definitive sediment toxicity test	☐	☐	☐
Warning Chart	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)**	☐	☐	☐
	Prepared for each reference toxicant and continually updated **(Must)**	☐	☐	☐
	Within acceptable warning limits (± 2 SD on log scale)	☐	☐	☐
	LC50 for survival within the warning limits (± 2 SD) of the historic reference toxicant mean	☐	☐	☐
Sample Handling				
Sample Collection	Multiple field replicates (ie: separate samples from different grabs or cores taken at the same site) be taken at each sampling station, including 1 or more reference stations	☐	☐	☐
	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, oxidation-reduction potential and T° be measured in the field to help characterize the sample	☐	☐	☐
	Care to be taken to minimize loss of fines during sample collection **(Must)**	☐	☐	☐
	Sample T° upon receipt at lab be measured and recorded	☐	☐	☐
Volume	At least 5 to 7 L of sediment per sample is normally required; it is frequently necessary to combine subsamples to obtain the required sample volume	☐	☐	☐
Containers	Made of nontoxic material; either be 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	☐	☐	☐
Labeling	Immediately after filling, each sample container be sealed and labeled or coded **(Must)**	☐	☐	☐
	Labeling include at least a code or description which identifies sample type, source, precise location, replicate number, date of collection **(Must)**	☐	☐	☐
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 and are not allowed to dry **(Must)**	☐	☐	☐
	Samples stored for future use be held in airtight containers and in darkness at 4 ± 2 °C **(Must)**	☐	☐	☐
Holding Time	Test to be initiated within 6 weeks after sampling **(Must)**	☐	☐	☐
	Recommend test initiation within 2 weeks after sampling	☐	☐	☐
	Date of receipt of the sample(s) at lab to be recorded **(Must)**	☐	☐	☐
Subsample Storage	All stored subsamples are to be in darkness at 4 ± 2 °C **(Must)**; and held in sealed containers with no air space	☐	☐	☐
Subsample Mixing	Each subsample to be thoroughly remixed to ensure homogeneity before use **(Must)**	☐	☐	☐
Sample Handling	Has sediment sample handling guidance outlined in EPS 1/RM/29 been cited in lab's SOP?	☐	☐	☐

TEST SPECIFIC CHECKLIST

April 1999

Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges *C. tentans* or *C. riparius*

Parameter	Specification	Met Specifics? Y	N	NA
Spiked Sediment				
Mixture	A chemical/sediment mixture prepared by making up a stock solution of the chemical and then remixing one or more measured volumes into control sediment, in a manner resulting in a homogeneous distribution of the chemicals throughout the sediment	☐	☐	☐
Chemical	Chemical(s) to be tested be at least reagent grade	☐	☐	☐
Solvent	The preferred solvent for preparing stock solutions is test water	☐	☐	☐
	If an organic solvent is used, the test is to be 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 to be 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 to be used **(Must)**	☐	☐	☐
Mixing	The maximum conc. of solvent in the sediment is to be at conc. that does not affect the survival and growth of *Chironomus* sp. during the test	☐	☐	☐
	Mixing conditions are to be standardized for each treatment in a test **(Must)**	☐	☐	☐
	T° during mixing is to be kept low	☐	☐	☐
# Test Conc.	For a multi-conc. test, at least 5 conc. plus a control are to be prepared **(Must)**; 6 to 8 conc. plus a control are recommended	☐	☐	☐
# Replicates	A minimum of 5 replicates for each test conc. and each control sediment are to be prepared **(Must)**	☐	☐	☐
	Replicate controls be prepared and treated identically **(Must)**	☐	☐	☐
Endpoints	LC50 for mortality data and ICp for dry weight data **(Must)**; NOEC/LOEC are possible additional endpoints	☐	☐	☐
	The quantal mortality data conc. be used to calculate the 10d median LC50, together with its 95% confidence limits **(Must)**	☐	☐	☐
Storage	Once prepared, each mixture be placed in sealed container with no air space and stored in the dark at 4 ± 2 °C for 4 weeks before use in test	☐	☐	☐
	Other conditions are similar as those for definitive sediment toxicity test	☐	☐	☐
Test Report				
Sample Data	Description of sample type or coding, as provided to the lab personnel **(Must)**	☐	☐	☐
	Information on labeling or coding of each sample **(Must)**	☐	☐	☐
	Date of sample collection; data and time sample(s) received at lab **(Must)**	☐	☐	☐
Test Organism	Species and source of brood stock and test organisms **(Must)**	☐	☐	☐
	Instar, at start of test **(Must)**	☐	☐	☐
	For *C. tentans* only, mean & range of head capsule width at test start **(Must)**	☐	☐	☐
	Any unusual appearance 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 of test water **(Must)**	☐	☐	☐
	Measured characteristics of test water, before and/or at start of test **(Must)**	☐	☐	☐
Test Method	Citation of biological test method used **(Must)**	☐	☐	☐
	Design and description if specialized procedure or modification of standard test method **(Must)**	☐	☐	☐
	Brief description of frequency and type of observations and 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 the test method document **(Must)**	☐	☐	☐
	# of discrete samples per treatment; # of replicate test chambers for each treatment; # and description of treatments in each test including the control(s); test concentrations if applicable **(Must)**	☐	☐	☐

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Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges *C. tentans* or *C. riparius*

Parameter	Specification	Met Specifics? Y	N	NA
Test Conditions (con't)	Depth/volume of sediment and overlying water in each test chamber **(Must)**	☐	☐	☐
	# of organisms per test chamber and treatment **(Must)**	☐	☐	☐
	Test option chosen (static or daily renewal) **(Must)**	☐	☐	☐
	Feeding regime and ration **(Must)**	☐	☐	☐
	Indication of any aeration of overlying water (including rate) **(Must)**	☐	☐	☐
	Dates when test was started and ended **(Must)**	☐	☐	☐
	For each sample, all measurements of sediment particle size, % water content, and TOC; and of porewater pH and ammonia **(Must)**	☐	☐	☐
	For at least one test chamber representing each treatment, all measurements of temperature and D.O. in overlying water, made at start of test and three or more times per week thereafter; all measurements of conductivity, pH and ammonia in overlying water made at start and end of test **(Must)**	☐	☐	☐
	For each treatment, mean ± SD for % of midge larvae that survived the 10d exposure; mean ± SD for dry weight of surviving midge at test end; results of any statistical comparisons **(Must)**	☐	☐	☐
	CV for mean % survival and mean individual dry weight of replicate control groups at test end **(Must)**	☐	☐	☐
Test Results	Any LC50 (with 95% confidence limits and if calculated, the slope) determined **(Must)**	☐	☐	☐
	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 **(Must)**	☐	☐	☐
	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 **(Must)**	☐	☐	☐
	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 **(Must)**	☐	☐	☐
	Anything unusual about the test, any problems encountered, any remedial measures taken **(Must)**	☐	☐	☐
Info Kept On-File	Do lab SOPs indicate that the information on Section 7.2 of the EPS 1/RM/32 method must be kept on file for 5 years? **(Must)** For details of this information, see EPS 1/RM/32, section 7.2.	☐	☐	☐

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.