TEST SPECIFIC CHECKLIST

Revised: March 2005

Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test

Parameter	Specification	Met Specifics Y	N	NA
Sample Preparation				
Aeration	No aeration of samples, test concentrations, or filtrates	☐	☐	☐
pH Adjustment	No adjustment of sample pH **(Must)**	☐	☐	☐
Salinity Adjustment	No adjustment of sample salinity **(Must)**	☐	☐	☐
Colour/Solids/ Floatables	No adjustment or correction of light emission readings (e.g., using readings for concentrations of reference or other sediments **(Must)**	☐	☐	☐
Sieving	Samples are not wet sieved **(Must)**; large particles (i.e., ≥ 2 mm) should be removed using forceps/ gloved hands, or sample may be press-sieved (e.g., ≥2 mm screen)	☐	☐	☐
Homogenization	Any porewater separated from sample during transport and storage is remixed into the sample **(Must)**	☐	☐	☐
	Sample mixed until texture and colour are homogeneous	☐	☐	☐
	For each sample included in a test, mixing conditions (duration and T°) are to be as similar as possible **(Must)**	☐	☐	☐
	Immediately after mixing, samples are placed into labelled test chambers and into labelled containers for physicochemical analyses **(Must)**	☐	☐	☐
Temperature	Upon arrival at lab, T° and date of sample receipt are recorded **(Must)**	☐	☐	☐
Characterization	Each sample (including control and reference sediment, if used) is analyzed for whole sediment: % very coarse-grained sediment (i.e., particles >1.0 mm), % sand (i.e., particles >0.063 to 2.0 mm), % fines (i.e., particles ≤0.063 mm), % water content, and total organic carbon content; and for porewater: salinity and pH **(Must)**	☐	☐	☐
	Analyses of particle size distribution undertaken as soon as possible after sample collection **(Must)**	☐	☐	☐
	Porewater ammonia, salinity and pH measured within 24h of test if contribution of ammonia to sample toxicity is being investigated	☐	☐	☐
	Ammonia analyses conducted using standard procedures and calculations are based on test temperature and on the sample's porewater pH and salinity **(Must)**	☐	☐	☐
Subsamples for Moisture Content	3 replicates of 5.0 ± 0.2 g (precision, ± 0.01 g) dried at 100 ± 5 °C for 24 h	☐	☐	☐
Primary Dilution	7.00 ± 0.05 g whole, homogenized sediment in 35.0 mL dilution water, glass or disposable plastic beaker, mixed for 10 min on a magnetic stirrer with Teflon™ stir bar, at a rate such that the vortex depth is half the height of the liquid level	☐	☐	☐
Test Facilities, Equipment, and Supplies				
Test Facility	Clean laboratory with standard lighting	☐	☐	☐
	Well ventilated, free of fumes, and isolated from physical disturbances or airborne contaminants that might affect the test organisms **(Must)**	☐	☐	☐
	Isolated from areas where test sediments are prepared and removed from areas where equipment is cleaned	☐	☐	☐
Photometers	Microtox™ Model 500 Analyzer or equivalent temperature-controlled photometer (15 ± 0.5 °C for ≥15 cuvettes with test solutions; 5.5 ± 1 °C for 1 cuvette holding reconstituted bacteria in Reagent well) with reading light output at a wavelength of 490 ± 100 nm **(Must)**	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Facilities, Equipment, and Supplies (cont'd)				
Apparatus/Equipment.	Equipment and supplies which contact sediment or water must not contain substances that can be leached or dissolved in amounts that adversely affect the test organisms **(Must)**; and should be chosen carefully to minimize sorption of materials from water.	☐	☐	☐
	Equipment for performing the solid-phase test for sediment toxicity includes:			
	• Refrigerated water bath with temperature control (15 ± 0.5 °C).	☐	☐	☐
	• Test tube rack or incubator block for incubating test tubes containing concentrations of test material and *v. fischeri* in the water bath.	☐	☐	☐
	• Freezer (not self-defrosting or "frost free" type) for storing lyophilized bacteria (Bacterial Reagent).	☐	☐	☐
	• Pipettors for delivering volumes of 20, 500, 1000, and 1500 µL, with disposable plastic tips.	☐	☐	☐
	• Disposable polystyrene SPT tubes (15.5 × 56 mm, 7.5-mL capacity, hemispherical bottom) or equivalent test tubes.	☐	☐	☐
	• Disposable glass cuvettes (borosilicate, 3-mL capacity, 50 mm length × 12 mm diameter, flat bottom).	☐	☐	☐
	• Disposable filter columns for SPT or equivalent test tubes.	☐	☐	☐
	• volumetric borosilicate glassware (acid washed) for processing small aliquots of samples.	☐	☐	☐
	• Countdown timer or stopwatch.	☐	☐	☐
	• Magnetic plate mixer with Teflon™ stir bar.	☐	☐	☐
	• A balance, accurate to 0.01 g.	☐	☐	☐
	• A drying oven (100 ± 5 °C).	☐	☐	☐
	• Weighing vessels for dry weight determination.	☐	☐	☐
	• Metal spoon or spatula for sample homogenization.	☐	☐	☐
Cleaning Procedure.	All equipment and supplies that might contact test sediment or water must be clean and dry **(Must)**.	☐	☐	☐
	All nondisposable materials should be washed after use..	☐	☐	☐
	Recommended cleaning procedure: Soak; detergent wash; 2 tap water rinses; acid wash (e.g., 10% nitric or hydrochloric acid) to remove scale, metals, and bases; 2 rinses with deionized water; pesticide-free acetone wash to remove organic compounds and hexane wash for oily residues; 3 rinses with high-quality deionized water (Note: Please advise labs that Environment Canada has added an additional step to the cleaning procedure in more recently published methods. In between "hexane wash for oily residues" and "3 rinses with high quality deionized water" add "allow organic solvent to volatilize and rewash with detergent if necessary". This step had been since hexane is not water soluble and would not be removed with 3 rinses of deionized water.). . .	☐	☐	☐
Test Conditions				
Test Species..	*Vibrio fischeri* (formerly classified as *Photobacterium phosphoreum*); strain NRRL B-11177 **(Must)**.	☐	☐	☐
Test Type.	Static..	☐	☐	☐
Aeration.	None **(Must)**	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Conditions (continued)				
Reconstitution Solution	Pure, non-toxic distilled or deionized water, used to activate Bacterial Reagent **(Must)**	☐	☐	☐
Control/Dilution Water (Diluent)	Purchased or taken from laboratory supply (confirm that laboratory supply does not decrease light production by *V. fischeri*)	☐	☐	☐
	3.5% NaCl solution used for diluting each sample of test sediment **(Must)**	☐	☐	☐
Reconstitution of Bacterial Reagent	Lyophilized bacteria (Bacterial Reagent) reconstituted with Reconstitution Solution; swirl 3-4 times, empty into disposable glass cuvette, mix 10 times with 0.5 mL pipette and held at 5.5 ± 1°C	☐	☐	☐
	Record time of Bacterial Reagent reconstitution **(Must)**	☐	☐	☐
Test T° and Incubations	Reconstituted bacterial solution used within 3 hours of reconstitution **(Must)**; recommend using within 2 hours	☐	☐	☐
	V. fischeri held at 5.5 ± 1.0 °C once reconstituted until aliquots are transferred to each test concentration **(Must)**	☐	☐	☐
	Test concentrations equilibrate to 15 ± 0.5°C for ≥10 minutes before inoculation with bacterial solution **(Must)**	☐	☐	☐
	Test sediment concentrations (and reference sediment, if included) incubated at 15 ± 0.5°C for 20 minutes after inoculation with bacterial solution **(Must)**; filter columns inserted into tops of SPT tubes above surface of test concentration	☐	☐	☐
	Following incubation and filtration, 500 µL filtrate transferred to cuvettes and held at 15 ± 0.5°C for 10 minutes for stabilization before light output is measured **(Must)**	☐	☐	☐
# Conc.	12 test concentrations; 3 replicate control solutions (Diluent only), prepared using a large bore pipette	☐	☐	☐
	Maximum test concentration normally 197 000 mg/L (19.7%, wet wt:vol); 0.5 dilution series	☐	☐	☐
Inoculation	20 µL of reconstituted bacterial reagent into each test concentration **(Must)**; mixed 3 times with 1.5 mL pipette	☐	☐	☐
	Timing of inoculation should match timing of transfer of filtrates to cuvettes and reading of luminescence (i.e., ≤4 min)	☐	☐	☐
# Replicates/Conc.	Only 1 replicate per test conc. required; 3 replicates for control **(Must)**	☐	☐	☐
Observations	Cuvettes placed in photometer read well; light levels of all test filtrates and controls measured	☐	☐	☐
Endpoint	Mean (± SD) of light readings for control solutions **(Must)**	☐	☐	☐
	IC50 (mg/L) for inhibition of light emission, calculated by software or manually; normalized for moisture content of sediment (i.e., calculated on dry-weight basis) **(Must)**	☐	☐	☐
Interim Guidelines for Judging Toxicity				
	Are guidelines being used appropriately?	☐	☐	☐
Guideline # 1	Any test sediment from a particular sampling station and depth is judged to have failed this sediment toxicity test if the IC50 is <1000 mg/L, regardless of grain size characteristics			
Guideline # 2	For any test sediment from a particular sampling station and depth which is comprised of <20% fines and has an IC50 of ≥1000 mg/L, the IC50 of this sediment must be compared against a sample of "clean" reference sediment or negative control sediment (artificial or natural) with % fines content that does not differ by more than 30% from that of the test sediment. Based on this comparison, the test sediment is judged to have failed the sediment toxicity test if, and only if each of the following two conditions apply: - its IC50 is more than 50% lower than that determined for the sample of reference sediment or negative control sediment; and - the IC50s for the test sediment and the reference sediment or negative control sediment differ significantly			

Parameter	Specification	Met Specifics Y	N	NA
Test Organisms				
Source	Strategic Diagnostics Inc.	☐	☐	☐
Age	Uniform strain of lyophilized bacteria ("Bacterial Reagent") harvested during the exponential phase of growth.	☐	☐	☐
Storage	Bacterial Reagent stored at constant temperature, within -20 °C and -25 °C, until expiry date provided by supplier.	☐	☐	☐
Lot #	Lot # and expiry date of bacteria used in each test recorded **(Must)**.	☐	☐	☐
QA/QC				
Validity Criterion	Test invalid if the coefficient of variation (CV) of the mean light readings measured for the filtrates of the 3 control solutions is >12% **(Must)**.	☐	☐	☐
Reference Toxicity Test	Perform within 1 month of each solid-phase sediment toxicity test and upon first use of a new batch of Bacterial Reagent using a suitable positive control sediment and the procedures and conditions described for measuring the toxicity of test sediment **(Must)**.	☐	☐	☐
	Determine IC50 for light emission and 95% confidence limits.	☐	☐	☐
	Reference toxicant can be a standard contaminated sediment or a spiked control sediment.	☐	☐	☐
	≥5 reference toxicity tests conducted with different lots of Bacterial Reagent using the same reference toxicant to determine intra laboratory precision (expressed as %CV).	☐	☐	☐
	Has sediment spiking guidance outlined in EPS 1/RM/30 been cited in lab's SOP?	☐	☐	☐
Warning Chart	Prepared for each reference toxicant and continually updated by plotting IC50s derived from reference toxicity tests successively on the warning chart **(Must)**.	☐	☐	☐
	Each new IC50 compared with established limits of the chart **(Must)**.	☐	☐	☐
	The logarithm of the concentration (including IC50) should be used in calculations and plotting.	☐	☐	☐
Positive Control Sediment	Recommended for inclusion in each series of toxicity tests; can be a standard contaminated sediment, a spiked control sediment, or a highly contaminated sample of field-collected sediment, previously shown to be toxic to *V. fischeri*.	☐	☐	☐
Reference/Negative Control Sediment	Included in any test series involving ≥ 1 sample of coarse-grained test sediment (i.e., <20% fines) **(Must)**.	☐	☐	☐
	% fines content of reference sediment does not differ by more than 30% from that of the test sediment **(Must)**.	☐	☐	☐
	Should be included in each test series.	☐	☐	☐
	May be clean field-collected sample or artificial negative control sediment formulated in the lab.	☐	☐	☐
	Field-collected negative control sediment collected from ≥1 site where geochemical properties of sediment, including grain size characteristics, are similar to test sediment, (ideally from a clean site in the general vicinity of the test sediment).	☐	☐	☐
	Artificial negative control sediment prepared in laboratory using kaolin clay and/or washed silica sand with grain sizes matching those of test sediment(s). If sediments in a test series contain a wide range of % fines, then more than 1 negative control sediment with range of % fines included in test.	☐	☐	☐
Controls	Run in triplicate; consists of Diluent plus Reconstituted Reagent (bacteria).	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Sample Handling				
Sample Collection	≥5 field replicates (i.e., separate samples from different grabs or cores taken at the same time) to be taken from each sampling station and depth of interest.	☐	☐	☐
	≥5 samples taken from each reference station (recommend ≥1 reference station).	☐	☐	☐
	A benthic grab or core sampler used rather than a dredge.	☐	☐	☐
	Care taken during sampling to minimize loss of fines **(Must)**.	☐	☐	☐
	Detailed records of field and sampling conditions should be maintained by sediment sample collectors.	☐	☐	☐
Containers.	New or thoroughly cleaned and rinsed with clean water **(Must)**.	☐	☐	☐
	Filled to exclude air..	☐	☐	☐
Labelling.	Sealed and labelled or coded immediately after filling **(Must)**.	☐	☐	☐
	Labelling includes at least a code which identifies sample type, source, precise location, replicate #, and date of collection **(Must)**, name and signature of sampler	☐	☐	☐
Sample Holding Time.	Test to be initiated within 6 weeks after sampling **(Must)**; recommend test initiation within 2 weeks; or preferably within 1 week.	☐	☐	☐
Holding Conditions	Upon collection, warm (>7 °C) samples are to be cooled to 1 - 7 °C (with ice or frozen gel packs), and kept in the dark and cool (4 ± 3 °C) during transport.	☐	☐	☐
	Samples to be kept from freezing, partially freezing or drying out during transport and storage **(Must)**.	☐	☐	☐
	Samples stored for future use are held in airtight containers and in the dark at 4 ± 2 °C **(Must)**.	☐	☐	☐
Sample Volume.	~100 mL.	☐	☐	☐
Subsample Storage.	Subsamples stored for future analyses are stored in the dark at 4 ± 2 °C **(Must)**; in sealed containers with no air space..	☐	☐	☐
Subsample Mixing.	Subsamples thoroughly re-mixed immediately prior to analyses **(Must)**.	☐	☐	☐
Sample Handling.	Has sediment sample handling guidance outlined in EPS 1/RM/29 been cited in lab's SOP?.	☐	☐	☐
Test Report				
Sample Data.	Brief description of sample type or coding as provided to the laboratory personnel **(Must)**.	☐	☐	☐
	Information on labelling or coding of each sample **(Must)**.	☐	☐	☐
	Date of sample collection **(Must)**.	☐	☐	☐
	Date sample(s) received at test facility **(Must)**.	☐	☐	☐
Test Organisms.	Species and strain **(Must)**.	☐	☐	☐
	Lot number and expiry date **(Must)**.	☐	☐	☐
Test Facilities & Apparatus.	Name and address of test laboratory **(Must)**.	☐	☐	☐
	Name of person(s) performing the test **(Must)**.	☐	☐	☐
	Name and Model # of *Analyzer* (photometer) used for measuring light emissions **(Must)**.	☐	☐	☐
Reconstitution Solution and Solid-Phase Diluent.	Type and source **(Must)**.	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Report (continued)				
Test Method.	Citation of biological test method used (i.e., EPS 1/RM/42) **(Must)**.	☐	☐	☐
	Name and citation of program(s) and methods used for calculating statistical endpoints **(Must)**.	☐	☐	☐
Test Conditions and Procedures.	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in EPS 1/RM/42 **(Must)**.	☐	☐	☐
	Number of discrete samples per treatment **(Must)**.	☐	☐	☐
	Number of replicates (if any) for each treatment **(Must)**.	☐	☐	☐
	Number and description of treatments in each test including the control solution(s), positive control sediment(s), and field-collected reference sediment(s) **(Must)**.	☐	☐	☐
	Date when the test was performed **(Must)**.	☐	☐	☐
	For each sample: % very coarse-grained sediment, % sand, % fines, % water content, total organic carbon, porewater salinity, porewater pH, and porewater ammonia **(Must)**.	☐	☐	☐
	Indicate if any samples of test sediment (including reference sediment) were press-sieved to remove large particles and/or detritus or indigenous organisms, including the procedure and mesh size used if applied **(Must)**. . . .	☐	☐	☐
	Appearance (colour, turbidity) of filtrates in cuvettes for each treatment **(Must)**.	☐	☐	☐
Test Results.	Light readings (mean, SD, CV) for replicate control solutions **(Must)**.	☐	☐	☐
	any IC50s and their 95% confidence limits, with method of calculation and units (mg/L), expressed to three significant figures **(Must)**.	☐	☐	☐
	All statistical results for "pairwise" or other comparisons of endpoint values **(Must)**.	☐	☐	☐
	A statement as to whether or not a test sediment is judged to be toxic, including a description of the guidelines used to reach that judgement **(Must)**.	☐	☐	☐
	Results for each IC50 (including its 95% confidence limits) with the reference toxicant(s) determined using the same lot of Bacterial Reagent as that used in the sediment toxicity test, determined within one month of the test and when the lot was first tested; together with the geometric mean value (± 2 SD) for the reference toxicant(s) as derived previously at the laboratory **(Must)**.	☐	☐	☐
	Anything unusual about the test, any deviation from the procedures and conditions of EPS 1/RM/42, any problems encountered, and any remedial measures taken **(Must)**.	☐	☐	☐
Original Data Sheets.	Original data sheets must be signed or initialled, and dated by the laboratory personnel conducting the tests **(Must)**.	☐	☐	☐
Info. Kept on-File	Do lab SOPs indicate that the information on Section 7.2 of the EPS 1/RM/42 method must be kept on file for ≥5 years? **(Must)**.	☐	☐	☐
	For details of this information, see Section 7.2 of EPS 1/RM/42.			