2. SOLID-PHASE TEST FOR SEDIMENT TOXICITY USING THE LUMINESCENT BACTERIUM, *VIBRIO FISCHERI*

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1. Objective and scope of the method

The solid-phase Microtox™ test for measuring the toxicity of whole sediment using luminescent bacteria (*Vibrio fischeri*) is best run as part of a battery of toxicity tests to estimate the toxic potential of sediment. The endpoint of the test can be used as part of a sediment quality assessment. Because the test is relatively inexpensive, rapid and easy to run, it can be used on its own to screen large numbers of samples, in order to delineate the spatial extent of sediment contamination.

The test system is automated and the bacterial reagent is supplied in a lyophilized (freeze-dried) form, so there is no need for time-consuming culture of the test organisms. As supplied, they are ready for testing at any time that samples might arrive.

The test is most commonly applied to the assessment of freshwater, estuarine, or marine sediment, and to terrestrial soils, but is theoretically applicable to any similar solid material, such as sludges and ore concentrates.

2. Summary of the test procedure

The solid-phase test for measuring the toxicity of whole sediment samples using luminescent bacteria is summarized in Table 1.

Table 1. Rapid summary of the test procedure.

Test organism	*Vibrio fischeri*, strain NRRL B-11177
Type of test	20 minute static test
Test vessels	Disposable glass cuvettes
Volume of test solution	1.5 mL
Inoculum	20 µL reconstituted bacteria
Lighting	Does not apply
Test temperature	15 ± 0.5°C
Experimental configuration	12 test concentrations and three control solutions
Endpoints	Moisture corrected IC50 in mg/L (dry-weight basis), others possible, *e.g.,* IC25
Measurements	Light levels of all test filtrates and controls measured
Reference toxicants	Toxic positive control sediment (*e.g.*, certified reference sediment)

The procedure involves the following steps:

- preparation of the primary dilution of the sediment;
- preparation of sample serial dilutions in diluent using a 50 % dilution series (Fig. 1);

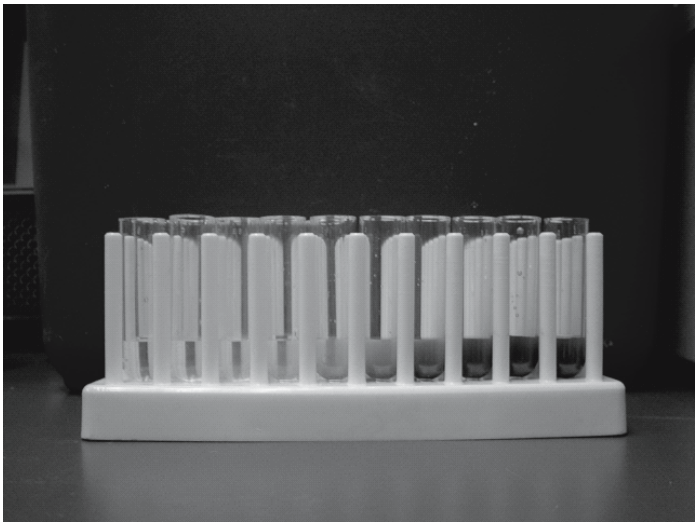

Figure 1. Preparation of whole sediment sample concentrations by serial dilutions in water using a 50 % dilution series.

Figure 2. M500 Microtox photometer, solid-phase tubes, and the computer to run the test, calculate the endpoints, and store the data.

- mixing the dilutions with an inoculum of test organisms (reconstituted *V. fischeri*) and incubation for 20 minutes in test tubes held in a water bath at 15 ± 0.5°C;
- filtration of the contents of each test tube;
- stabilization of the filtrate at 15 ± 0.5°C for 10 minutes in a series of cuvettes held within wells of a photometer;
- photometric reading of light produced by the luminescent bacteria remaining in the filtrate (Fig. 2).

The test array consists of 3 controls (comprised of dilution water only) and 12 test concentrations. The maximum test concentration is 197,000 mg/L (19.7%, wt:v), with each successive concentration being 50% of the previous one. A schematic overview of the various stages in the test is shown in Figure 3.

3. Overview of applications reported

A test for measuring the toxicity of aqueous samples to luminescent bacteria was developed in the late 1970s (Anonymous, 1979). Researchers began applying this test to measure the toxicity of contaminated sediments in the 1980s and 1990s by assessing effects in solvent extracts (Schiewe et al., 1985; True and Heyward, 1990) and sediment porewaters (True and Heyward, 1990; Giesy et al., 1988). The different solvent systems, which can be used for the extraction of contaminants from sediments, have different efficiencies, and can even prove toxic to the bacteria (Tay et al., 1992). Furthermore, solvent extracted contaminants will not necessarily represent the bioavailable contaminants. Therefore, a Direct Sediment Toxicity Testing Procedure for measuring sediment toxicity using luminescent bacteria was introduced by Canadian researchers (Brouwer et al., 1990), and an acute Solid-Phase Test for sediment (or soil) toxicity was subsequently adopted and standardized by Microbics Corporation (Carlsbad, CA), as one of several Microtox test methods (Microbics, 1992). Kwan and Dutka (1995) compared these two solid-phase toxicity test methods, and confirmed their suitability as sensitive tests to detect bioavailable toxicants in solid-phase samples. Both tests are practical, reproducible, rapid, and relatively inexpensive compared to solid-phase extraction procedures. There are currently a number of Solid-Phase Test methods using luminescent bacteria, which are in use internationally and have been compared in detail (Environment Canada, 2002).

Since its introduction, the test has been widely utilized. It was employed in correlation studies between a number of solid-phase sediment toxicity tests and *in situ* benthic community structure in freshwater and marine sediments (Day et al., 1995; Porebski et al., 1999; Zajdlik et al., 2000). It can be used to assess the toxicity of sediment being considered for disposal at sea, on land or at any freshwater, estuarine, or marine sites where regulatory appraisals or stringent testing procedures apply. The test has been used to assess the quality of contaminated soils (Qureshi et al., 1998; Environment Canada, unpublished data) and freshwater sediments (Day et

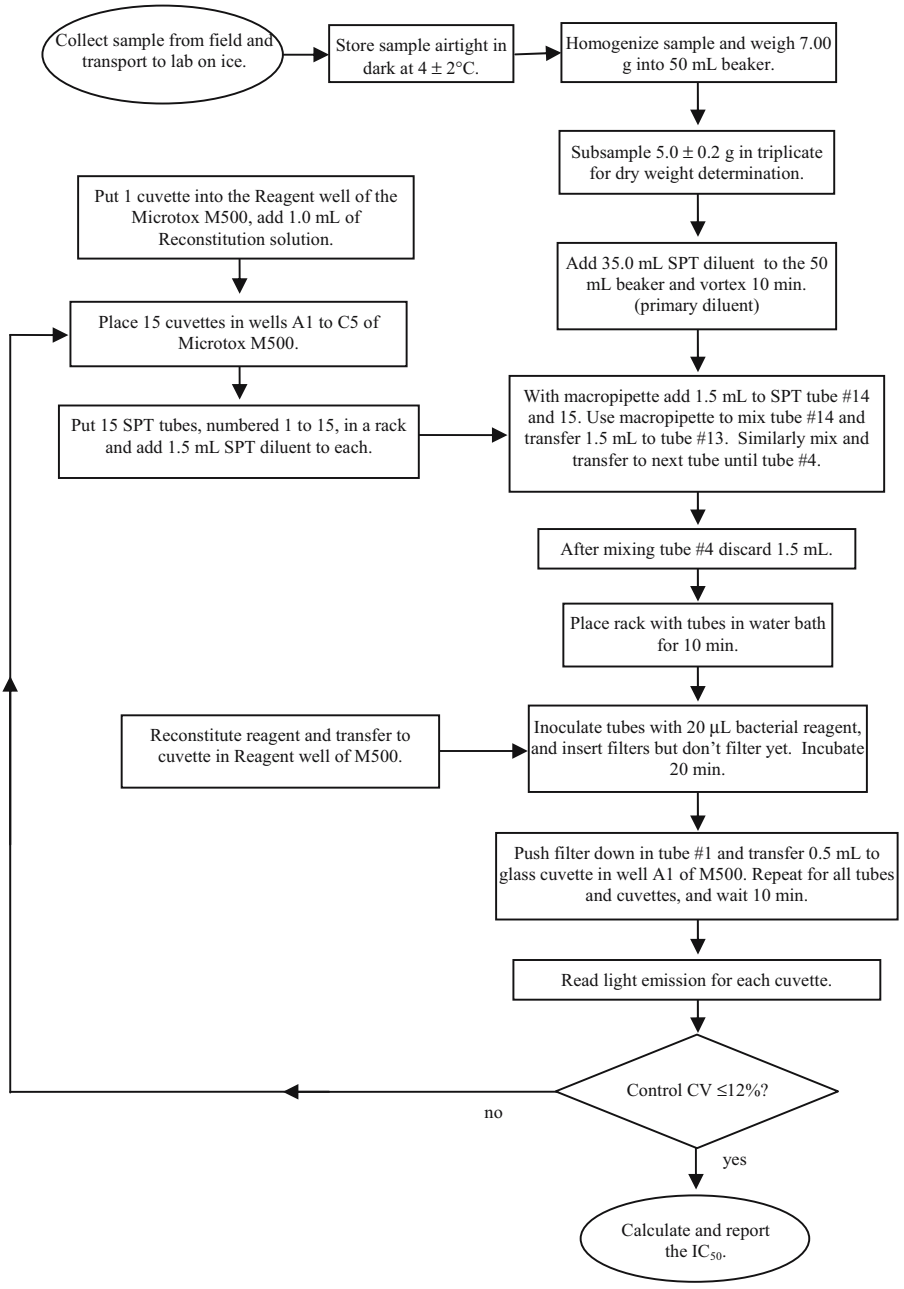

Figure 3. Summary of the Solid-Phase Test using luminescent bacteria.

al.,1995; Carter et al., 1998; Denning et al., 2003). Test design and data interpretation were studied by Ross and Leitman (1995) and Ringwood et al. (1997). Toxicity appraisals of harbour sediments (Halifax, Canada) have been conducted (Tay et al. 1992; Cook and Wells, 1996), and the role of this and other assays in assessing sediment toxicity was examined by Ross (1998) and Bombardier and Bermingham (1999). The interlaboratory precision of a solid-phase test for sediment toxicity using *V. fischeri* was studied by Ross et al. (1999) and McLeay et al. (2001). Mueller et al. (2003) used the assay to determine the effectiveness of sediment bioremediation techniques.

Since its introduction, the test has been widely used by North-Americans and other researchers and regulators for evaluating the toxicity of sediments. Environment Canada (1992) recommended the use of the Microtox™ *solid-phase* test method (Microbics, 1992) for evaluating the toxicity of solid media, while recognizing that the standardization of the test method was in its infancy. In 2002, after marked research efforts and inter-laboratory validation testing, Environment Canada published a Reference Method for determining the toxicity of sediment using luminescent bacteria, *Vibrio fischeri* (Environment Canada, 2002).

This chapter is based on the Environment Canada published Reference Method (Environment Canada, 2002), and describes the procedure for conducting solid-phase tests for measuring sediment toxicity using the luminescent bacterium *Vibrio fischeri*. Further details can be obtained by consulting the Reference Method, which represents one of several regulatory biological test methods recommended as part of sediment assessment under the Canadian Environmental Protection Act Disposal at Sea Regulations (Environment Canada, 1997a; CEPA, 1999; Government of Canada, 2001).

Several organizations have published methodology guideline documents or laboratory standard operating procedures for the solid-phase test for sediment toxicity using luminescent bacteria in which pre-test, test conditions and procedures are summarized: ASTM (1995), Microbics (1995), Environment Canada (1996a), AZUR (1997), AZUR (1998a; 1998b), Environment Canada (1999a), and NICMM (1999). The test has been included as part of several recent reviews concerning sediment toxicity testing (Johnson 1998; Burton et al., 2003).

4. Advantages of conducting the Solid-Phase Test using luminescent bacteria

The solid-phase test for measuring sediment toxicity with the luminescent bacterium *V. fischeri* has several advantages over the more conventional sediment tests used to assess the toxicity of contaminated sediments with invertebrates such as chironomids, amphipods, molluscs, polychaetes, and echinoderms:

- test rapidity: the solid-phase tests using luminescent bacteria can be completed in hours as opposed to days for the other sediment tests;
- small sample volume requirements: this feature makes samples easy to collect and cheaper to ship to testing laboratories. Sample volumes of 25 – 50 mL suffice to

conduct several replicate tests and to measure moisture content of the sample. Other tests might require one L or more of sediment;

- freeze-dried bacterial reagent: no time consuming culturing of test organisms is required since test organisms (*V. fischeri*) are lyophilized and available commercially. Field-collection and continuous culturing of other light-producing micro-organisms would require considerable efforts;
- repeatability: a high quality control source of reagents and supplies ensures standardization and repeatability of results worldwide;
- inter-laboratory validation: the test was validated by inter-laboratory studies conducted by Ross et al. (1999) and McLeay et al. (2001). The latter study involved six testing laboratories where 19 samples were analyzed using the procedure described in this chapter. The inter-laboratory precision was "very favorable and well within the limits considered acceptable in other studies of this nature";
- realistic hazard assessment: test results have shown statistically significant correlation with contaminant concentrations, benthic community structure, and many conventional invertebrate whole sediment bioassays, as discussed by Day et al. (1995) and Zajdlik et al. (2000);
- versatility: the same test procedure can be conducted with (freshwater, estuarine and marine) sediments and soils;
- relevance: bacteria form the basis of many important ecosystem functions such as biodegradation of organic matter, nutrient recycling, etc. They therefore represent an important group of organisms for inclusion in any battery of toxicity tests.

5. Test species

The recommended test organisms come from a standardized culture (strain NNRL B-11177, Northern Regional Research Laboratory, Peoria, IL, USA), and belong to a particular species of luminescent marine bacteria (*i.e.*, *Vibrio fischeri*, formerly classified as *Photobacterium phosphoreum*). This is a bacterium which normally lives in the ocean, and produces blue-green light on a continual basis by a series of enzymatic reactions utilizing metabolic energy obtained from the electron transport system if sufficient oxygen is available (Environment Canada, 1992).

6. Culture/maintenance of organisms in the laboratory

Standard cultures of *V. fischeri* can be purchased from Strategic Diagnostics Inc.[1] Bacteria are marketed as a uniform strain of lyophilized (*i.e.,* freeze-dried under vacuum) bacteria ("*Bacterial Reagent*"), harvested during the exponential phase of

[1] This and related products and disposal supplies for performing solid-phase toxicity tests using *V. fischeri* were formerly marketed by AZUR Environmental Ltd. (Carlsbad, CA). Marketing rights for Microtox™ products and reagents have now been acquired by Strategic Diagnostics Inc. in Newark, DE. For contact information, see their web site at www.sdix.com, or phone 800 544-8881. The web site lists international distributors for approximately 60 countries.

growth. Production lots are sold in packages containing ≥10 sealed vials. Each vial harbors about 100 million lyophilized organisms. Each lot is suitable for at least two hours (Environment Canada, 1992) and for up to three hours of testing (Gaudet, 1998), after bacteria have been reconstituted to an active state. Because the bacterial reagent (lyophilized *V. fischeri*) is available commercially, there is no time consuming culturing of test organisms. The reagent is stored frozen until required. If desired, the *V. fischeri* can be cultured in the laboratory using methods outlined in ISO (1993).

The number and expiry date of bacterial lots used in each toxicity test should be recorded and this information should be included in the test-specific report together with the species and strain of the test organism. It is recommended that other data specific to the test organisms, including their source, date of receipt, and temperature during storage or holding, should either be included in the test-specific report or held on file for a minimum of five years.

7. Preparation of bacteria for toxicity testing

The bacteria ("*Bacterial Reagent*") are sold in packages containing sealed vials of lyophilized organisms that are stored frozen until use. Once a vial is opened, it is reconstituted by quickly pouring the *Reconstitution Solution* held at 5.5 ± 1°C in a cuvette placed into the reagent well of the *Model 500 Analyzer* or other photometer (see Section 8.7) into the vial. After swirling three times, vial contents are poured back into the same cuvette. The reconstituted bacteria are then held in the reagent well at 5.5 ± 1°C and are ready for use.

8. Testing procedure

8.1 FACILITIES

The test can be conducted in a normal, clean laboratory with standard lighting. The need for any special facilities would be governed by the degree of hazard associated with the samples that are to be tested, and by the risk of sample and apparatus contamination. Facilities must be well ventilated, free of fumes, and isolated from physical disturbances or airborne contaminants that might affect the test organisms.

8.2 APPARATUS AND SUPPLIES

A list of apparatus and supplies required for the test are provided below:

- A Microtox™ *Model 500 Analyzer*[2] or equivalent temperature-controlled photometer (15 ± 0.5°C for ≥15 cuvettes with test solutions; 5.5 ± 1°C for

[2] These items are available from Strategic Diagnostics Inc.

single cuvette holding reconstituted bacteria in *Reagent* well) capable of reading light output at a wavelength of 490 ± 100 nm.
- A refrigerated water bath with temperature controlled at 15 ± 0.5°C.
- A test tube rack or incubator block for incubating tubes containing concentrations of test material and *V. fischeri* in the water bath.
- A 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).[2]
- Disposable glass cuvettes (borosilicate, 3 mL capacity, 50 mm length × 12 mm diameter, flat bottom).[2]
- Disposable filter columns for SPT test tubes.[2]
- Freeze-dried *Bacterial Reagent*.[2]
- *Reconstitution Solution* (purified non-toxic water).[2]
- Solid-Phase *Diluent* (non-toxic distilled or deionized water plus 3.5% sodium chloride).[2]
- Volumetric borosilicate glassware (acid washed) for processing small aliquots of samples.
- A countdown timer or stopwatch.
- A magnetic plate mixer with Teflon stir bar.
- 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.

The *Bacterial Reagent* should remain in a freezer at -20°C until used. Similarly, Solid-Phase *Diluent* and *Reconstitution Solution* should be stored at room temperature until required.

8.3 MANIPULATIONS, ADJUSTMENTS, AND CORRECTIONS

- Test sediments must not be wet-sieved, and no adjustments of porewater salinity are permitted. Sample pH must not be adjusted. No aeration of samples, test concentrations, or filtrates should be performed.
- Light-emission readings for concentrations of each test material must not be adjusted or corrected.
- The statistical endpoint for the test (*i.e.*, IC50) must be normalized for the moisture content of the sample.

8.4 TEMPERATURE

- The *Bacterial Reagent* is reconstituted to an active state in non-toxic distilled or deionized water and held at 5.5 ± 1.0°C until aliquots are transferred to each

test concentration. Normally this temperature is met by placing the cuvette with the reconstituted bacterial solution in the specified well of the photometer if a Microtox™ *Model 500 Analyzer* is used. Otherwise a temperature-controlled incubator must be used for this purpose.

- All concentrations of test material inoculated with bacteria must be incubated for 20 minutes at 15 ± 0.5°C. The temperature is allowed to stabilize for 10 minutes prior to inoculation by *Bacterial Reagent*. A temperature-controlled water bath or room would serve this purpose.
- Following incubation and filtration, all test solutions transferred to cuvettes must be held at 15 ± 0.5°C during the subsequent 10-minute period for stabilization of the filtrates. This temperature control is normally achieved within the wells of the photometer. Alternatively, the cuvettes containing test filtrates can be held within this temperature range in a cuvette holder placed in a temperature-controlled incubator or room.

8.5 TIMING OF EVENTS

- The lyophilized bacteria should be reconstituted immediately before inoculating the test concentrations. This bacterial solution should be used within 2 h, and within a maximum of 3 h after reconstitution. The time of reconstitution should be logged on a bench sheet.
- A primary dilution of sediment is prepared by stirring the sediment and diluent for ten minutes. Then, aliquots are removed to prepare test concentrations. The latter must be allowed to equilibrate to 15 ± 0.5°C for a minimum of 10 minutes before inoculation with bacterial solution. Inoculation should proceed as quickly as possible; all test concentrations should be inoculated within a total time span of ≤ 4 minutes. Record the time of the first inoculation as the start of the test.
- All test concentrations must be incubated for 20 minutes after inoculation of the first tube with bacteria. Once the test concentrations are filtered and transferred to cuvettes, the filtrates must be incubated in cuvettes for 10 minutes in the temperature-controlled wells of the photometer before their light output is measured.
- Total elapsed time for the transfer of filtrates to cuvettes and for reading luminescence of the test filtrates should be similar to that spent inoculating the test concentrations with bacteria (≤ 4 min).

8.6 CONDUCTING THE TEST

The procedures to be followed when performing a solid-phase test involve the simultaneous incubation of three control solutions (comprised of an inoculum of reconstituted *V. fischeri* in Solid-Phase *Diluent*) together with 12 different concentrations of each sample of test material in Solid-Phase *Diluent*. After a prescribed incubation period, the solutions (held in test tubes at a controlled temperature) and test concentrations are filtered, and the resulting filtrates are

transferred to cuvettes. After a brief period for stabilization of holding conditions for the filtrates, the light production by test organisms remaining in each filtrate is measured by a photometer. Table 2 provides a checklist of the conditions, apparatus, and procedures recommended for conducting the test. Figure 3 is a flowchart of the entire test procedure.

The procedures applied herein to a photometer assume the use of a Microtox™ *Model 500 Analyzer* or another photometer with similar features. Since the Microtox™ *Model 500 Analyzer* has 30 wells for holding cuvettes containing filtrates of test concentrations, the laboratory analyst using this photometer has the option of performing two tests simultaneously on different test materials. Note that the option to analyze two test materials (*i.e*., two samples) simultaneously is recommended to save time and *Reagent*.

The following sections describe the procedure by which a sample of test material is processed for assessment of its toxic potential.

8.7 PHOTOMETER, WATER BATH, AND BENCH SHEET

- Switch on the computer, photometer, and balance.
- For the *Model 500 Analyzer,* ensure that the temperature selector switch at the back is set to "Microtox Acute".
- Place 15 cuvettes in the first 3 rows (A-C) of wells. These will be maintained at 15 ± 0.5°C. The incubated wells are arrayed in a grid of rows labeled A to C and columns numbered 1 to 5. They are referenced as A1 to C5 (Fig. 4).
- Place one cuvette in the reagent well, and pipette 1.0 mL of *Reconstitution Solution* into it. This will be maintained at 5.5 ± 1°C.
- Switch on the water bath incubator. Allow the water temperature to stabilize at 15 ± 0.5°C.
- Stir the sample to homogenize it, using a stainless steel spoon.
- Place 15 SPT tubes into a rack.

8.8 SUBSAMPLES FOR MOISTURE

- For each test sediment, label and weigh three empty weighing dishes, and record the weights to the nearest 0.01 g.
- Add 5.0 ± 0.2 g of sediment to the vials and record the weights to the nearest 0.01 g.
- Dry the subsamples by putting the vials into an oven at 100 ± 5°C for 24 h. Record the oven temperature.
- Record the dry weights to the nearest 0.01 g.

Table 2. Checklist of required or recommended test conditions and procedures.

Facilities and equipment	Photometer (*e.g.* Microtox™ *Model 500 Analyzer*, available from Strategic Diagnostics Inc.) reading light output at 490 ± 100 nm; incubator for single cuvette holding reconstituted bacteria at 5.5 ± 1°C; for ≥ 15 cuvettes at the test temperature (15 ± 0.5°C), in an incubator or controlled-temperature room.
Reconstitution Solution	Non-toxic distilled or deionized water or *Reconstitution Solution* supplied by Strategic Diagnostics Inc.
Control/dilution	Solid-Phase *Diluent* (3.5% NaCl solution).
Test temperature	15 ± 0.5°C.
Color correction	No correction.
Aeration	None required.
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 test material in 35 mL Solid-Phase *Diluent*, 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 concentrations	Maximum test concentration normally 197,000 mg/L (19.7% wet wt:vol) with two-fold dilutions, for a total of 12 test concentrations in disposable polystyrene tubes; three control solutions (*Diluent* only); left for 10 min to equilibrate to the test temperature.
Test species	*Vibrio fischeri*, strain NRRL B-11177 (Note: the use of lyophilized *Bacterial Reagent* supplied by Strategic Diagnostics Inc. would increase the standardization of the test procedure), reconstituted by swirling each vial 3-4 times, emptied into a disposable glass cuvette, mixed 10 times with 0.5 mL pipette and held at 5.5 ± 1°C.
Inoculum	20 µL into each test concentration, mixed 3 times with 1.5 mL pipette.
Incubation	20 min at test temperature, filter columns inserted into tops of SPT tubes above surface of test concentration.
Filtrate transfer	500 µL into disposable glass cuvettes at test temperature, left 10 min for equilibration.
Observations	Set light level with first control and then insert cuvettes into photometer read well; light levels of all test filtrates and controls are then measured.
Endpoint	IC50 (mg/L), calculated by software used by each laboratory; normalized for moisture content of sediment (*i.e.*, calculated on dry-weight basis).

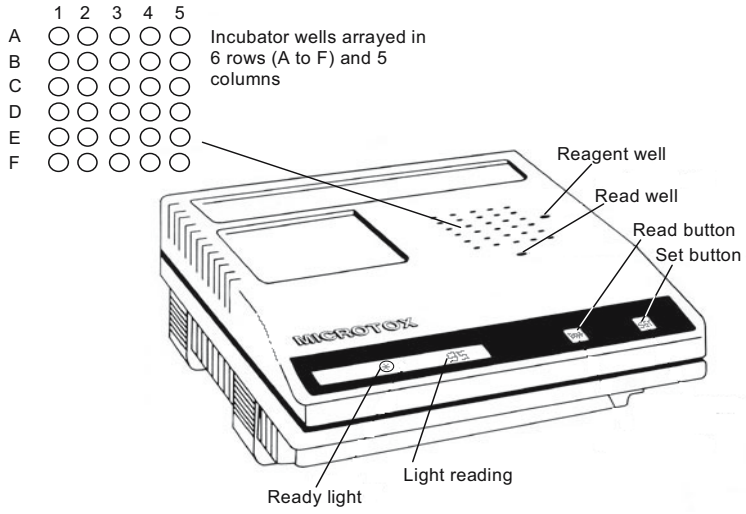

Figure 4. Microtox M500 analyzer showing the operational features and the array of incubator wells into which are placed the glass cuvettes.

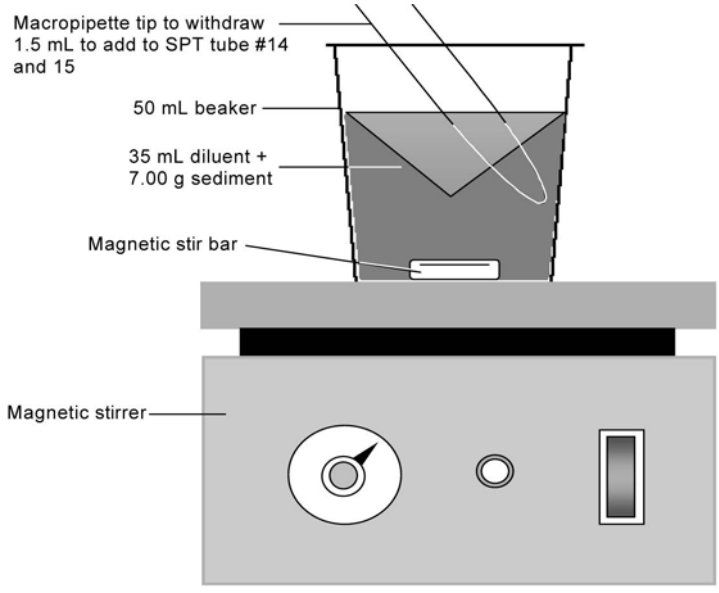

Figure 5. The primary sample dilution is prepared on a magnetic stirrer, agitated for 10 min at a rate such that the vortex is half the height of the liquid level. To aspirate 1.5 mL, the macropipette tip is inserted near the side of the beaker, at about half the depth of the stirring sample.

8.9 PRIMARY DILUTION

- Weigh 7.00 ± 0.05 g of homogenized subsample into a glass or disposable 50 mL plastic beaker.
- Add a 2.5 cm Teflon-coated magnetic stir bar and 35 mL of Solid-Phase *Diluent* to the beaker.
- Stir for 10 min on a magnetic stirrer at a rate to create a vortex that reaches 1/2 the height of the liquid level (Fig. 5).

8.10 TEST CONCENTRATIONS

- Dispense 1.5 mL Solid-Phase *Diluent* into the first 14 tubes in the rack. Tube 15 will be the highest concentration, taken from the primary dilution.
- Following the 10-minute stirring of the primary dilution, use a large-bore macro pipette tip to transfer 1.5 mL of sample suspension from the 50 mL beaker, while it is still stirring, to each of SPT tubes 15 and 14. To do this, insert the pipette tip near the side of the beaker, at about half the depth of the stirring sample. Avoid plugging the tip while aspirating the sample (Fig. 5).
- Beginning at SPT tube 14, which now has 3 mL of solution, make 1:2 serial dilutions as follows. Mix the contents 3 times with the macro pipette, and then quickly draw up a volume of 1.5 mL from about one third depth (to help draw some of the heavier sand grains). Transfer this aliquot to tube 13. Repeat this mixing and transferring from tube 13 to 12, and continue consecutively thereafter from tube 12 to 11, tube 11 to 10, etc. until 1.5 mL of the 1:2 serial dilutions is transferred into tube 4. Finally, discard 1.5 mL from tube 4. Tubes 1-3 contain *Diluent* only, and serve as the controls (Fig. 6).
- Place the rack with SPT tubes containing all test concentrations (including controls) into the water bath at 15 ± 0.5°C. Leave it undisturbed for 10 min for temperature equilibration. The water level of the bath should be just above the liquid level in the SPT tubes.

8.11 RECONSTITUTION OF BACTERIAL REAGENT

- Take a vial of freeze-dried bacteria (Microtox™ *Acute Reagent*) from freezer storage.
- Open the vial and reconstitute its contents by quickly pouring the Reconstitution Solution from the cuvette in the reagent well of the *Model 500 Analyzer* (or other photometer) into the vial, swirling three times and pouring the rehydrated bacteria into the same cuvette.
- Replace the cuvette in the reagent well.
- Using a 500 µL pipette, aspirate any remaining *Reconstituted Bacterial Reagent* from the vial, add it to the cuvette, and mix 10 times using the same pipette and tip.
- Record the reagent lot number, expiry date, and time of reconstitution on the bench sheet.

8.12 INOCULATION AND INCUBATION

- Prepare the following three pipettes: (a) a repeat pipette (such as an Eppendorf or Oxford Nichiryo) with a 0.5 mL syringe fitted with an ultra micro tip; (b) a macro pipette (*e.g*. Oxford 1-5 mL); and (c) a 500 µL pipette.
- Following the 10-min temperature equilibration of the SPT tubes containing the test concentrations set a timer for 20 min but do not start it.
- Make sure the repeat pipette is set to dispense 20 µL per ejection. Place the tip below the surface of the reconstituted bacteria (*Reconstituted Bacterial Reagent)*, and draw up sufficient reconstituted bacteria for at least 18 ejections.
- Holding the tip above the *Reagent* and against the cuvette wall, eject 2 times. Wipe the tip with a clean wiper.

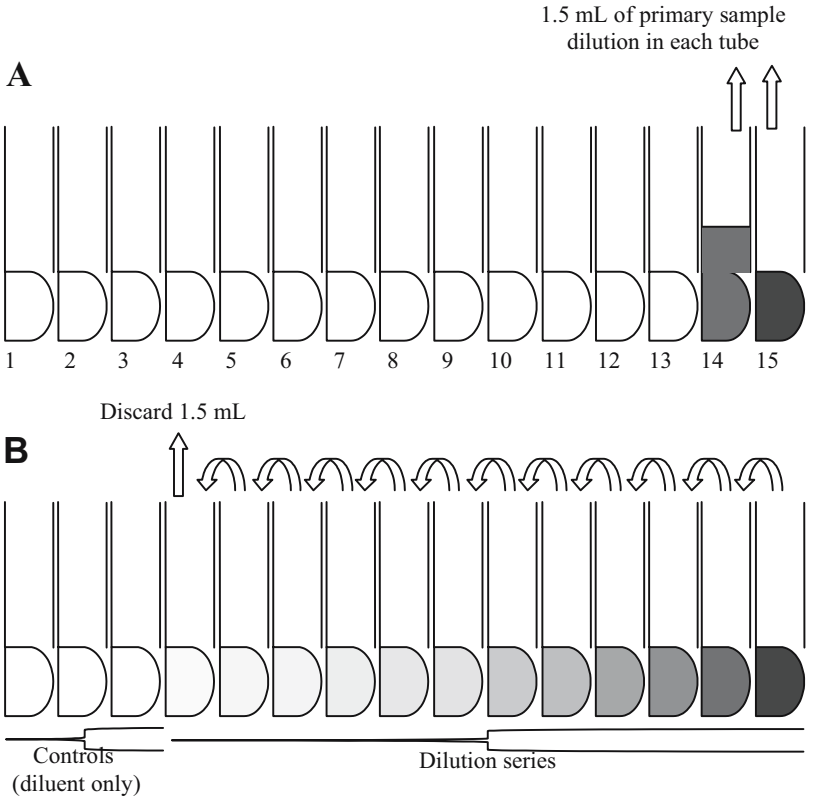

Figure 6. SPT tubes are placed in a rack, then (A) SPT diluent (1.5 mL) is added to each SPT tube (#1 to 15) and 1.5 mL of the primary sample dilution from the beaker on the vortex mixer is added with a macropipette to tubes 14 and 15. The solution in tube 14 is mixed with the macropipette (B) and 1.5 mL is transferred each time to the next tube and mixed. Finally, 1.5 mL is discarded from tube #4.

- Start the 20-min timer, record the time as the "Start" of the test on the bench sheet, and immediately eject 20 μL of *Reagent* into each of the SPT tubes, starting with tube 1 (first control solution) and continuing consecutively to and including tube 15. Resting the collar of the ultra micro tip on the top edge of each tube will assure the tip will be at the surface of the sample and not on the bottom of the tube.
- Remove and discard the ultra micro tip. Eject the remaining *Reagent* from the syringe into the holding cuvette in the reagent well.
- If a second test (*i.e.*, with a second test material) is to be performed concurrently using the remaining 15 wells of the *Analyzer*, refit the syringe with a clean tip, refill it with Bacterial Reagent, and inoculate the next series of test concentrations.
- With the macro pipette set at 1.5 mL, mix each tube twice, beginning with tube 1 (first control) and proceeding consecutively through to and including tube 15. (If performing 2 tests consecutively replace tip between samples).
- Insert a filter column in each tube, with its lower end positioned ~1 cm above the surface of the liquid. Do not wet the filter, since this might adversely affect filtration of the sample.

8.13 PREPARING THE COMPUTER

- Prepare the computer to receive data from the photometer.
- Start the appropriate software program and menus for the solid-phase test.
- Follow the on-screen instructions.
- Refer to the user's manual for additional information.
- Information requested by the software might include the number of controls (3), number of dilutions (12), initial concentration (197,000 mg/L), dilution factor (2), and the test time (10 min).

8.14 FILTRATION AND LIGHT LEVEL READINGS

- When the 20-min timer sounds, respond to the computer software as appropriate.
- Reset the timer for 10 min and start it. If you are using SDI software, this period is automatically initiated. Otherwise, program the computer software to initiate this 10-min period automatically at the touch of a key.
- Gently push the filter in tube 1 (first control) down far enough to obtain slightly greater than 500 μL of filtrate in the filter column. Then, using the 500 μL pipette, transfer 500 μL of filtrate to the cuvette in well A1 of the photometer.
- Repeat this step for all 15 tubes using the same pipette tip, ending with 500 μL of filtrate being transferred from tube 15 to cuvette C5.
- Take note of the time required to complete all the transfers. Respond to the prompt by the software (assuming that this is a component of that used).

- Often with sediments having a high proportion of fines the filter in the highest test concentration (tube 15) will become plugged. Obtain what can be recovered, and transfer it to the designated cuvette (C5). Usually the luminescence from these samples will have decreased to zero before you test the highest concentration. Make a note of such problems on the bench sheet.
- After 10 minutes, set the light level with the first control cuvette and then read light production taking approximately the same amount of time as was taken to filter and transfer the filtrate to the cuvettes. Timing is prompted by the software if SDI software is used.
- The data are then sent from the photometer to the computer, and received by the software which builds a data file.

The procedure to measure light production of the bacteria in the test concentrations will vary depending on the photometer and software used. If a *Model 500 Analyzer* is used, place the first control (cuvette A1) into the read well and press the "set" button. The instrument lowers the cuvette into the well (sometimes 2 or 3 times) to set the zero (dark) and control reading at about 95 and thereby establishes the appropriate sensitivity range for light measurements. A green "ready" light will appear. Then press the "read" button. After reading the cuvette, remove it from the read well and replace it in the incubator block. Proceed to read and record the light emission from all the cuvettes, taking approximately the same average time per cuvette as was required to do the filtering and transferring. Using SDI software, this timing is performed by the computer and prompts occur which indicate when each cuvette should be read.

9. Post-exposure observations/measurements and endpoint determinations

Much of the guidance in this section is based on the document "Solid-Phase Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria (*V. fischeri*)" (Environment Canada, 2002). Additional discussion has been provided where necessary to expand on certain concepts.

9.1 DATA ANALYSIS

The mean and standard deviation of the light readings for the control solutions used in the study must be calculated. These values are used to determine the coefficient of variation of the mean for the control solutions, which is used as one of the criteria described herein for judging if the test results are valid (Section 10). The coefficient of variation of the light readings for the control solutions must be $\leq$ 12 % for a particular test to be considered valid and for data analysis to proceed. A study performed according to this method should include one or more samples of test sediment together with one or more samples of reference sediment. Additionally, the inclusion of one or more samples of positive control sediment for use as a reference toxicant is required as a measure of sensitivity of the bacterial reagent. Grain size analysis must be performed on each test and reference sediment. Tests involving one

or more samples of coarse-grained sediment (*i.e*., sediment with < 20% fines) must also include one or more samples of negative control sediment (artificial or natural) or clean reference sediment with a percentage of fines content that does not differ by more than 30% from that of the coarse-grained test sediment(s). In each instance, the statistical endpoint to be calculated for each of these test materials is the ICp (inhibiting concentration for a specified percent effect). Unless specified otherwise by regulatory requirements or by design, the endpoint for this method is the concentration causing 50% inhibition of light, *i.e*., the IC50. The calculations to estimate the IC50 and its 95% confidence limit are included in the most recent (1999 or later) *Omni*™ software packages (Version 1.18 or equivalent) formerly marketed by AZUR Environmental Ltd. and now available from Strategic Diagnostics Inc. (see footnote 1for contact information). In 1999, AZUR released an operating software termed "*MicrotoxOmni*". This software includes a calculation option called "*AutoCalc*". The "*AutoCalc On*" option must be selected if this software is applied; otherwise, erroneous results might occur. *AutoCalc* selects only the data points around the IC50 that have a range of $0.02 < Gamma < 200$. Using these data points, all the various contiguous series are examined by an iterative convergence regression analysis to determine the tightest 95% confidence limits. If the *MicrotoxOmni* software is not available, guidance for estimating IC50 (together with its 95% confidence limit) is provided in Environment Canada (1992), and other statistical software packages are available which enable this calculation by linear regression (Environment Canada, 1997b; 1997c; 2003).

For each test concentration, *Gamma* (Γ; see definition in the Glossary) is calculated (ASTM, 1995) as:

$$\Gamma = (I_c/I_t) - 1 \qquad (1)$$

where: I_c = the average light reading of filtrates of the control solutions, and I_t = the light reading of a filtrate of a particular test material concentration. Values for each test filtrate that fall within the range of $0.02 < Gamma < 200$ are plotted. The IC50 is the concentration that corresponds to a *Gamma* of 1. If the *MicrotoxOmni* software is not available, data entered for linear regression should be checked against the observed readings to guard against errors in entry and anomalous estimates of IC50. A manual plot and its estimated IC50 could also be used to check any computer-generated graph and the computer calculation of the IC50 (Environment Canada, 1992). Plots of *Gamma* and % effect versus concentration generated by the *MicrotoxOmni* software are shown in Figure 7 from a solid-phase test on a field-collected sediment sample, and the raw data from the test are provided in Table 3.

A linear regression of logC (concentration, on the ordinate) *versus* log Γ (on the abscissa) is computed according to the following equation (ASTM, 1995):

$$\log \Gamma = b(\log C) + \log(a) \qquad (2)$$

In the above equation, 'b' is the slope and 'log(a)' is the intercept of the regression line with the ordinate (y-axis) at log C = 0. To determine the IC50, solve equation 2 for C, when Γ = 1.

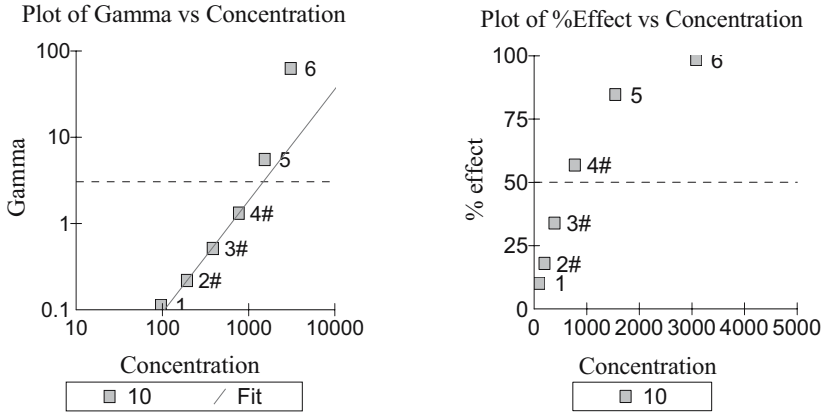

Figure 7. Plots of Gamma and % effect versus concentration, as plotted by the Microtox Omni *software. These plots are from the raw data provided in Table 3 that were also generated using the* Microtox Omni *software from a test performed on a field-collected sediment.*

Table 3. Raw data used in Figure 7 plots. "It" is the light reading of a filtrate of a particular test material concentration. The MicrotoxOmni *software automatically selects the data to be used in the calculation.*

Test treatment	***Concentration (mg/L)***	***It***	***Gamma***	***% Effect***
Control	0.000	103.37		
Control	0.000	100.32		
Control	0.000	94.86		
1	96.19	89.50	0.1119 #	10.07
2	192.4	81.70	0.2181 #	17.90
3	384.8	65.78	0.5129 #	33.90
4	769.5	42.99	1.315 #	56.80
5	1539	15.28	5.513 #	84.65
6	3078	1.56	62.79 #	98.43
7	6156	0.68	145.3 *	99.32
8	12310	0.32	310.0 *	99.68
9	24630	0.26	381.8 *	99.74
10	49250	0.09	1105 *	99.91
11	98500	0.08	1243 *	99.92
12	197000	0.07	1421 *	99.93

- used in calculation; * - invalid data.

The IC50 and its associated values for the 95% confidence limits must be converted to (and expressed as) mg/L on a dry-weight basis. This is achieved using the dry-weight data (see Section 8.8) (ASTM, 1995). The IC50 (as well as the upper and lower value of the confidence limits) of the wet sediment is multiplied by the average ratio of the dry-to-wet subsample weights:

$$IC50 = IC50_w \times [(S1_d/S1_w) + (S2_d/S2_w) + (S3_d/S3_w)]/3 \quad (3)$$

where: $IC50_w$ is the calculated IC50 (or its 95% confidence limits) of the wet sediment sample, $S1_d$ through $S3_d$ are the dry weights of the sediment subsamples (from Section 8.8), and $S1_w$ through $S3_w$ are the corresponding wet weights. These calculations can be expedited by entering the weights and IC50 values into a spreadsheet and using the necessary formulae.

Investigators should consult Environment Canada (2003) for detailed guidance regarding appropriate statistical endpoints and their calculation. The objectives of the data analysis are: to quantify contaminant effects on test organisms exposed to various samples of test sediment; to determine if these effects are statistically different from those occurring in a reference sediment; and to reach a decision as to sample toxicity (Section 10). Initially, ICp (normally, IC50) is calculated for each sample (including those representing the field-collected reference sediment).

Depending on the study design and objectives, an appropriate number (typically, ≥ 5/station, for each depth of interest) of replicates of field and reference sediments should be collected and evaluated. Each series of toxicity tests must include a minimum of three replicate control solutions, and one or more test sediments.

10. Factors capable of influencing performance and interpretation of results

Interpretation of results is not necessarily the sole responsibility of the laboratory personnel undertaking the test. This might be a shared task which includes an environmental consultant or other qualified persons responsible for reviewing and interpreting the findings.

Environment Canada (1999b) provides useful advice for interpreting and applying the results of toxicity tests with environmental samples; and should be referred to for guidance in these respects. Initially, the investigator should examine the results and determine if they are valid. In this regard, the criterion for a valid test must be met (CV ≤ 12 %). Additionally, it is recommended that the dose-response curve for each sample of test sediment be examined to confirm that light loss decreases as test concentration decreases, in an approximately monotonic manner. We recommend that an r^2 value for the regression equation (provided by the *MicrotoxOmni* software, or calculated by the user) be ≥ 0.90. If not (*e.g.*, if one or more data points appear to be "out of place" with respect to the others), consideration should be given to repeating the test for that sample as this type of response is normally caused by pipetting errors. Finally, the results of any reference toxicity test (Environment Canada, 1990; 1995) with a toxic positive control

sediment, which was initiated with the same lot of *Bacterial Reagent* as that used in the sediment toxicity test, should be considered during the interpretive phase of the investigation. These results, when compared with historic test results derived by the testing facility using the same reference toxicant and test procedure (*i.e.*, by comparison against the laboratory's warning chart for this reference toxicity test), will provide insight into the sensitivity of the test organisms as well as the laboratory's testing precision and performance for a reference toxicity test with *V. fischeri*. If the results of the reference toxicity test are outside of three standard deviations of the historic mean value of all previous tests with this reference toxicant (the "Control Limit"), all test conditions pertaining to the test should be double-checked thoroughly and consideration should be given to repeating the test (Fig. 8).

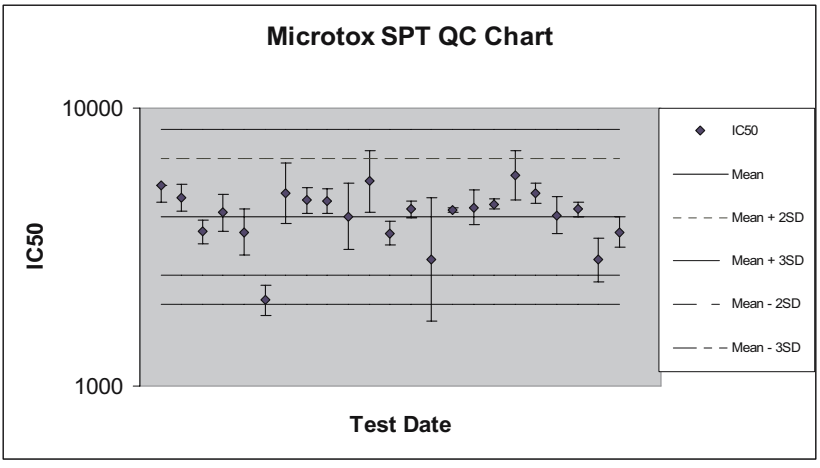

Figure 8. Example of a Quality Control chart for the Microtox Solid-Phase Test.

All data representing the known physico-chemical characteristics of each test material (including that for any samples of reference sediment or negative and positive control sediment included in the study) should be reviewed and considered when interpreting the results. The analytical data determined for whole sediment should be compared with the known influence of these variables on light production by *V. fischeri*, and also compared with sediment quality guidelines for the parameters measured.

Concentrations of porewater ammonia and/or hydrogen sulphide can be elevated in samples of field-collected sediment. This might be due to organic enrichment from natural and/or anthropogenic (man-made) sources. The known influence of ammonia (see, for example: Qureshi et al., 1982; Tay et al., 1998; McLeay et al., 2001) indicates that it is not a major confounding factor in this test. The known influence of hydrogen sulphide (Jacobs et al., 1992; Brouwer and Murphy 1995;

Tay et al., 1998) on the inhibition of light production by *V. fischeri* should be considered together with measured concentrations of these variables in porewater, when considering and interpreting results for field-collected samples and reference sediments.

Observations of turbid or highly colored filtrates analyzed for light emission by *V. fischeri* should be considered when reviewing and interpreting the test results.

A number of variables besides toxicity can interfere with readings of light production by *V. fischeri* surviving in the filtrate of each test concentration, and thus can confound the interpretation of the test results. Investigators performing this method, as well as those interpreting the findings, should be aware of these confounding factors and their implications in terms of judging if test materials are toxic or not. Variables which can interfere with the light production of *V. fischeri* in test filtrates include the following (ASTM, 1995; Ringwood et al., 1997; Tay et al., 1998):

- Sorption of *V. fischeri* to sediment particles (particularly fine-grained ones) retained on the filter; and the resulting loss of transfer of these luminescent bacteria to the filtrate.
- Sorption of *V. fischeri* to the filter, and the resulting loss of transfer of these luminescent bacteria to the filtrate.
- Optical interference of the filtrate, due to color (light absorption) and/or turbidity (light scatter).

The grain size of test sediments can be a significant confounding factor, since an increasing percentage of clay in the test material has been demonstrated to cause a proportionate decrease in resulting IC50s determined for *V. fischeri* recovered in filtrates of uncontaminated sediment. Samples of uncontaminated sediment comprised primarily of sand-sized particles (*e.g.*, 0-5% fines) characteristically yield an IC50 of 28,000 to >100,000 mg/L in a *V. fischeri* solid-phase assay (Cook and Wells, 1996; Ringwood et al., 1997; Tay et al., 1998). IC50s show a "precipitous drop" (Benton et al., 1995; Ringwood et al., 1997; Tay et al., 1998) when the percentage of fines in uncontaminated sediment increases from 5 to ~ 20%, whereupon the IC50 might possibly range from 5000 to 15,000 mg/L depending on the nature of the fines (*e.g.*, % clay and % silt) (Ringwood et al., 1997; Tay et al., 1998; McLeay et al., 2001). Higher percentages of fines in uncontaminated sediment typically show a "leveling off" of further declines in IC50s associated with increasing sediment fines. *V. fischeri* solid-phase tests with 100% kaolin clay have reported IC50s ranging from 1,373 to 2,450 mg/L (Ringwood et al., 1997; Tay et al., 1998). In an interlaboratory study to validate this Environment Canada's Reference Method, IC50s for a sample of 100% kaolin clay ranged from 1,765 to 2,450 mg/L (McLeay et al., 2001). Together, these findings support the Environment Canada interim guidelines for judging samples as toxic or not, according to the *V. fischeri* solid-phase assay (Environment Canada, 2002). These guidelines take into account the percentage of fines in the test sediment and the known sharp inflection of values when their fines content reaches or exceeds 20% (Ringwood et al., 1997), as well as the ability of a test material comprised of 100%

clay to reduce the IC50 to as low as 1,765 mg/L using this reference method (McLeay et al., 2001).

The two following interim guidelines are used for judging the toxicity of test sediment samples. The first one, which has been recommended and applied by Environment Canada in the past (Environment Canada, 1996b; Porebski and Osborne, 1998), is based on the premise that all samples are toxic, according to this biological test method, if their IC50 is < 1,000 mg/L, regardless of grain size characteristics. The second guideline is based on the premise that samples with < 20% fines might be toxic at an IC50 ≥ 1,000 mg/L, since confounding grain size effects are appreciably less in coarse-grained sediment (Environment Canada, 2002).

The first interim guideline should be applied to all samples of test sediment with ≥ 20% fines, as well as to any sample with < 20% fines which has an IC50 < 1,000 mg/L. The second interim guideline should be applied to all samples of test sediment with < 20% fines that have an IC50 ≥ 1,000 mg/L. This second guideline enables toxic coarse-grained sediments to be identified as such when their IC50 is appreciably higher than 1,000 mg/L. It is recommended that the second interim guideline be applied to each sample of test sediment with < 20% fines, except in the instance where the IC50 is < 1,000 mg/L in which case the sample should be judged as toxic and the second guideline does not apply.

Guideline 1: any test sediment from a particular sampling station and depth is judged to have failed this sediment toxicity test if its IC50 is < 1,000 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 ≥ 1,000 mg/L. The IC50 of this sediment must be compared with a sample of "clean" reference sediment or negative control sediment (artificial or natural) with a % fines content that does not differ by more than 30% from that of the test sediment.[3] 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:

[3] The following two examples are provided to illustrate how this "must" criterion is to be applied when choosing a negative control sediment or reference sediment with a percentage of fines that does not differ by more than 30% from that of the test sediment. If the test sediment has a fines content of 10%, the percent fines of the reference sediment or negative control sediment must be within the range of 7 to 13%. Similarly, if the test sediment has a fines content of 5%, the percent fines of the reference or negative control sediment must be within the range of 3.5 to 6.5%.

(1) its IC50 is more than 50% lower than that determined for the sample of reference sediment or negative control sediment[4]; and

(2) the IC50s for the test sediment and the reference sediment or negative control sediment differ significantly.

The first condition for Guideline 2 is verified using the following examples for calculations as a guide: if the sample of reference or negative control sediment used to judge the toxicity of the course-grained test sediment has an IC50 of 20,000 mg/L, the IC50 of the test sediment must be < 10,000 mg/L. Similarly, if the sample of reference or negative control sediment used to judge the toxicity of the course-grained test sediment has an IC50 of 5,050 mg/L, the IC50 of the test sediment must be < 2,025 mg/L.

The second condition for Guideline 2 must be verified using a pairwise comparison of values for the two IC50s and their 95% confidence limits, which is described in Sprague and Fogels (1977) as a means of comparing two LC50s.[5]

11. Application of the Solid-phase Test using luminescent bacteria in a case study

In this section we will examine the application of the solid-phase tests for measuring sediment toxicity using the luminescent bacterium *V. fischeri* in a study of toxicity test responses along a known pollution gradient. The test responses will be compared to sediment contamination, other toxicity test responses, and the benthic community living at the test sites.

A study undertaken along a known pollution gradient was conducted in Sydney Harbour, Canada, and the relationships between sediment and porewater chemistry, benthic community structure, and biological toxicity tests were examined (Zajdlik et al., 2001). Major contaminants were PAHs, PCBs, and heavy metals. The toxicity tests employed were: whole sediment 10-day toxicity tests with four species of infaunal amphipods, 14-day survival and growth tests with two species of polychaetes, the solid-phase test for sediment toxicity using the luminescent bacterium *Vibrio fischeri*; and the echinoderm fertilization test on sediment porewater using three species of sea urchins.

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[4] This condition for judging sample toxicity was derived in light of the findings of two series of interlaboratory studies performed to validate this reference method (McLeay et al., 2001). In one series of tests with four identical subsamples of a contaminated sediment tested in separate assays by each of six participating laboratories, the lowest laboratory-specific IC50 was 14 to 48% lower (mean intralaboratory difference, 31%; n = 6) than its highest IC50. Given this degree of intralaboratory variability in IC50s for the same test sediment, as determined within individual laboratories, it is considered prudent to require that the IC50 for a sample of coarse-grained test sediment, if $\geq$ 1000 mg/L, must be more than 50% lower than that for the negative control or reference sediment with which it is compared, as one of the conditions for judging the sample as toxic.

[5] This pairwise comparison test delineated in Sprague and Fogels (1977) is thought to be suitable for comparing two IC50s (J.B. Sprague, pers. comm.). A more statistically rigorous pairwise comparison test for IC50s is currently under development by Environment Canada (Environment Canada, 2004).

A brief summary of the study results is presented in Table 4.

The luminescent bacterium solid-phase test performed well, and response (IC50) was negatively correlated with sediment contamination, and positively correlated with biological variables such as 10-day survival of infaunal amphipods in a whole sediment toxicity test, and with benthic community structure (average number of taxa per station). The mean PEL quotient for sediment contaminants (Long et al., 1998) was calculated. Results with mean PEL quotient values < 1 resulted in a 'pass' (*i.e.*, sediment sample was classified as non toxic using interpretation criteria presented in Section 10), while results yielding mean PEL quotient values > 1 resulted in a 'fail' (toxic response). This demonstrates that when established sediment quality guidelines predict an effect, the luminescent bacterium solid-phase test will show an effect, confirming its sensitivity and usefulness as a screening tool. Spearman rank correlation coefficient was used to test if the different biological toxicity tests used in the study ranked the stations in the same way. It was found that the luminescent bacterium solid-phase test correlated highly with the results of the 10-day amphipod sediment toxicity tests.

Table 4. Summary of selected results from Sydney Harbour, NS, Canada, pollution gradient study (Zajdlik et al., 2001).

Parameter	*Stations*				*Reference Station 12*	*Reference St. Ann's*
	1	*5*	*6*	*9*		
Sum of PAHs (µg/g)	212	86.9	36.9	3.18	0.45	0.37
Total PCBs (µg/g)	2.1	1.19	0.64	ND[a]	ND[a]	ND[a]
Lead (µg/g)	286	214	133	32	21	37
Zinc (µg/g)	516	866	281	91	56	84
Amphipod *(Amphiporeia virginiana)* % survival	3	52	53	74	79	77
Microtox IC50 (mg/L, moisture corrected)	97	123	145	1,010	13,200	1,730
PEL Quotient[b]	14.9	7.6	3.6	0.44	0.11	0.11
Average # Taxa	2.6	5.0	5.8	17	15.5	3

[a] ND = below detection limit; [b] PEL = Probable Effects Level (Long *et al.* 1998).

The study demonstrated the sensitivity relevance of the luminescent bacterium solid-phase test, since results correlated with sediment contamination, structure of

benthic community present at the sites, and with results of more traditional sediment toxicity tests such as the 10-day whole sediment toxicity tests undertaken with infaunal amphipods.

12. Accessory/miscellaneous test information

The Solid-Phase Test for sediment toxicity using luminescent bacteria was developed for testing samples of whole sediment and similar solid materials. The interlaboratory study described by McLeay et al. (2001) involved six testing laboratories and a total of 19 samples were analyzed using the procedure described in this chapter. The interlaboratory precision was within the limits considered acceptable in other studies of this nature (interlaboratory CV ranged from 12 % to 70 % for the 19 samples), and there was no relationship between laboratory experience with this toxicity test and the precision or validity of the results obtained. This indicates that the test is simple and reproducible. Cross-training new personnel with experienced personal, and close attention to quality control procedures, will ensure valid results.

The test is rapid (results for a sample can be obtained in slightly over an hour). Because two samples can be tested at one time, it is possible to test 6 to 8 samples in a normal working day, and this includes quality control procedures with reference toxicants and occasional duplicate assays. Therefore larger numbers of samples can be screened for toxicity using this test when compared with the traditional invertebrate toxicity test such as marine amphipods, freshwater chironomids, or soil invertebrates such as earthworms. Material costs per test are approximately 60 US$, and there are no labor costs for culturing the organisms as they are purchased in a ready-to-use form.

The small sample size required for the test (refer to Sections 8.8 and 8.9, and Figure 3), and the short test duration, make it easy for safe handling of test substances. Safety measures such as use of protective equipment (lab coat, gloves, goggles), and engineering controls (adequate ventilation and use of fume hoods), will allow the test to be carried out in a safe manner. It is necessary to have proper procedures in place for disposal of highly contaminated samples.

The procedures described in this chapter outline the endpoint for this method as the concentration causing 50% inhibition of light output, compared to unexposed control organisms (*i.e.*, the IC50 and its 95% confidence limit). There is no reason why an investigator could not choose to calculate an alternate more sensitive ICp value, such as an IC25. However, we would advise caution in using lower values of p such as an IC10 or an IC5, because these values may be within the normal variability of the test (Environment Canada, 2004).

13. Conclusions

The solid-phase test for measuring the toxicity of whole sediment using luminescent bacteria has been widely used for the assessment of freshwater, estuarine, or marine sediment, and terrestrial soils, but is theoretically applicable to any similar solid material, such as sludges and ore concentrates. The test is rapid, cost-effective, reproducible and correlated to in-situ toxic effects on benthic communities. Since the test provides information on potential toxic effects to bacteria, it is best used in a battery of toxicity tests (along with invertebrate toxicity tests) to estimate the toxic potential of sediment samples. The endpoint of the test can be used as part of an overall sediment quality assessment. Because the test is relatively cheap and easy to run, it can be used on its own to screen large numbers of samples, in order to delineate the spatial or temporal extent of sediment contamination.

The test is subject to a number of confounding factors, in particular the grain size of test sediments, and sulfide content of the sediments. Ammonia is not a strong confounding factor for this test, which could make the test useful for sediment toxicity identification evaluations. Interpretation criteria outlined in this chapter will provide guidance to the reader on the significance of the test results. More research is encouraged to validate or improve the interpretative guidance provided.

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Abbreviations

CV	coefficient of variation
ICp	inhibiting concentration for a (specified) percent effect
IC50	50% inhibiting concentration
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	polychlorinated-biphenyls
PEL	Probable Effects Level
SD	standard deviation
SOP	standard operating procedure
SPT	solid-phase test
wt:v	weight-to-volume.