Section F

TOXICITY TEST METHODS

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Acute Test Methods

Rainbow Trout 96 Hour Acute Lethality Test (Freshwater)

Parameter

Fish Acute Lethality

Test Method

Rainbow Trout Acute Lethality Test (≤10 ppt. salinity)

EMS Code

Species	Test	Units	EMS Code
Oncorhynchus mykiss (Rainbow Trout)	96hrLC20*	%(v/v)	0466 X068
	96hrLC50*	%(v/v)	**0461 X068**
	Pass/Fail*	% mortality	

*without pH adjustment

Additional EMS codes available upon request, for example to identify another species.

EC Test Methods

Biological Test Method: Acute Lethality Test Using Rainbow Trout, Report EPS 1/RM/9 July 1990 (with May 1996 and May 2007 amendments).

Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout, EPS 1/RM/13 Second Edition December 2000 (with May 2007 and February 2016 amendments).

Method Summary

Rainbow Trout are exposed in single or multiple concentration(s) of the sample and mortality is recorded over a period of 96 hours at 15±1°C. The pass/fail single concentration test uses undiluted material at 100% concentration and determines the percent mortality after 96 hours. The LC50 and LC20 tests use multiple concentrations of material diluted in laboratory water and determines the concentration that is lethal to 50% and 20% of the test fish respectively.

Applications

Industrial effluents (pulp mill and mining). Landfill and woodwaste leachates. Municipal wastewater. Agricultural runoff. Pure chemicals. Samples must have a salinity ≤10 ppt. The LC50 test is required for permitted discharges.

Sample Considerations

The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand, extremes in pH, extreme concentrations of suspended solids. Control/dilution water exhibiting extremes of water hardness or containing suspended solids, toxic chemicals or metals, may cause problems. Precautions must be taken to ensure proper handling of test organisms including proper acclimation, freedom from disease and previous prophylactic treatment.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Expel all air pockets. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

For an LC50 test 2–4, 20 L plastic cube-shaped containers or carboys are required. For a pass/fail single concentration tests 1–2, 20 L container(s) or carboy(s) are required.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 5 days from collection.

Endpoints

% Mortality, 96hr LC50, 96hr LC20

Quality Control

a) Reference toxicant warning chart data on test fish.
b) Negative control.
c) Routine chemistry of holding and dilution water.
d) Stock history of test fish.

Acceptability Criteria

Greater than 10% mortality or exhibition of atypical/stressed behaviour of the control fish renders the test invalid. The normal biological variation among individual fish also limits precision in a bioassay. Specific toxicity results are accurate only for the exact test parameters used, such as dilution, water hardness, and fish health conditions.

Interpretation

For the pass/fail single concentration and LC50 tests an effluent sample is considered to have failed if at 100% concentration, more than 50% of the test fish die after 96 hours of exposure. For the LC20 test an effluent sample is considered to have failed if at 100% concentration, more than 20% of the test fish die after 96 hours of exposure.

References

a) Environment Canada, Biological Test Method: Acute Lethality Test Using Rainbow Trout, Report EPS 1/RM/9 July 1990 (with May 1996 and May 2007 amendments).

b) Environment Canada, Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout, EPS 1/RM/13 Second Edition December 2000 (with May 2007 and February 2016 amendments)

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
December 31, 2000:	SEAM codes replaced by EMS codes. References updated. Clarification of pH adjustment. Units added.
June 23, 2017	BCELTAC Microbiology Toxicology Subcommittee

Rainbow Trout 96 Hour Acute Lethality Test with pH Stabilization (Freshwater)

Parameter

Fish Acute Lethality with pH Stabilization

Test Method

Rainbow Trout Acute Lethality Test with pH Stabilization for Wastewater Effluent

EMS Code

Species	Test	Units	EMS Code
Oncorhynchus mykiss (Rainbow Trout)	96hrLC20*	%(v/v)	
	96hrLC50*	%(v/v)	
	Pass/Fail*	% mortality	

*with pH adjustment

Additional EMS codes are available upon request, for example to identify another species.

EC Test Method

Biological Test Method: Procedure for pH Stabilization During the Testing of Acute Lethality of Wastewater Effluent to Rainbow Trout, EPS 1/RM/50 — March 2008. A revised version of this method has been developed specifically for pulp and paper effluents, EPS 1/RM/59, March 2018. This method is specific for the pH controller technique described in EPS 1/RM/50 as an add on to the acute fish toxicity test, EPS 1/RM/13.

Method Summary

Rainbow Trout are exposed in single or multiple concentration(s) of the sample and mortality is recorded over a period of 96 hours at 15±1°C. The pass/fail single concentration test uses undiluted material at 100% concentration and determines the percent mortality after 96 hours. The LC50 and LC20 tests use multiple concentrations of material diluted in laboratory water and determines the concentration of that is lethal to 50% and 20% of the test fish respectively. Parallel testing with and without pH stabilization is recommended to demonstrate the presence of ammonia toxicity.

Upward pH drift in the effluent test solution causes an increase in the concentration of un-ionized ammonia which is the most acutely toxic form present. The loss of CO_2 is caused by the standard test procedure of aerating the effluent sample with lab air. The pH stabilization is intended to replace the CO_2 lost during aeration in order to maintain the pH throughout the test at the same level found in the original sample. The pH stabilization is accomplished by using either the CO_2 injection or the pH controller techniques. The procedure is only applicable when used explicitly with conjunction to the reference method EPS 1/RM/13 “Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout”. The pH stabilization applies to both single and multiple concentration tests.

Applications

For use only with wastewater effluents. The LC50 test is required for permitted discharges.

Sample Considerations

Total ammonia must be measured in the sample and this measurement is used to determine if pH stabilization is appropriate. The procedure may only be used if the un-ionized ammonia present in the sample does not exceed 1.25 mg/L and it has been shown that a previously collected wastewater sample from the same source failed the Rainbow Trout acute lethality test following 1/RM/13.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Expel all air pockets. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume For an LC50 test 2–4, 20 L plastic cube-shaped containers or carboys are required. For pass/fail single concentration test 1–2, 20 L container(s) or carboy(s) are required.

Stability Store in dark at 4±2°C until ready for testing. Maximum Holding.Time, (MHT). = 5 days from collection.

Endpoints % Mortality, 96hr LC50, 96hr LC20

Quality Control

a) Reference toxicant warning chart data on test fish.
b) Negative control.
c) pH stabilized test can only be performed if the three conditions are met: 1) total ammonia must be measured, 2) failure of acute lethality reference method occurred, and 3) un-ionized ammonia concentration in the 100% test water does not equal or exceed 1.25 mg/L.
d) Stock history of test fish.

Acceptability Criteria Greater than 10% mortality or exhibition of atypical/stressed behaviour of the control fish renders the test invalid. A test is also considered invalid if a) the average pH in the 100% effluent test solution is greater than ± 0.2 units from the initial pH or b) the instantaneous pH in the 100% effluent test solution is greater than ± 0.3 units of the initial pH. The normal biological variation among individual fish also limits precision in a bioassay. Specific toxicity results are accurate only for the exact test parameters used, such as dilution, water hardness, and fish health conditions.

Interpretation For the pass/fail single concentration and LC50 tests an effluent sample is considered to have failed if at 100% concentration, more than 50% of the test fish die after 96 hours of exposure. For the LC20 test an effluent sample is considered to have failed if at 100% concentration, more than 20% of the test fish die after 96 hours of exposure.

If mortality occurs in the un-stabilized, but not the pH stabilized test, then acute lethality is due to increased levels of un-ionized ammonia as a result of upwards pH drift. If mortality results are similar in both the un-stabilized and the pH stabilized tests, then acute lethality is not due to un-ionized ammonia toxicity.

References

a) Environment Canada, Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout, EPS 1/RM/13 Second Edition December 2000 (with May 2007 and February 2016 amendments).

b) Environment Canada, Environmental Protection Series: Procedure for pH Stabilization During the Testing of Acute Effluents to Rainbow Trout, Report EPS 1/RM/50 — March 2008.

c) Environment Canada, Method Development and Applications Section, Environmental Science and Technology Centre, Supplementary Background and Guidance for Investigating Acute Lethality of Wastewater Effluent to Rainbow Trout, March 2008.

d) Environment Canada, Biological Test Method: Procedure for pH Stabilization During the Testing of Acute Lethality of Pulp and Paper Effluent to Rainbow Trout, EPS 1/RM/59, March 2018.

Revision History

July 2, 2008	Publication in the 2009 Laboratory Manual
June 23, 2017	BCELTAC Microbiology Toxicology Subcommittee

Marine Pacific Salmonid 96 Hour Acute Lethality Test (Seawater)

Parameter

Marine Pacific Salmonid Acute Lethality

Test Method

Marine Acute Lethality Test Using Pacific Salmonids (>10 ppt. salinity)

EMS Code

Species	Test	Units	EMS Code
Oncorhynchus kitsutch, (Coho)	96hrLC50	%(v/v)*	**0461 FA06**
	Pass/Fail	% mortality*	
Oncorhynchus tshawystcha, (Chinook)	96hrLC50	%(v/v)*	**0461 FA05**
	Pass/Fail	% mortality*	

*Lethality in 100% effluent concentration after salinity adjustment.

EMS codes will be assigned upon request for other related bioassay tests.

EC Test Methods

Biological Test Method: Acute Lethality Test Using Rainbow Trout, EPS 1/RM/9 July 1990 (including May 1996 and May 2007 amendments)

Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout, EPS 1/RM/13 Second Edition December 2000 (including May 2007 and February 2016 amendments)

Biological Test Method: Acute Lethality Test Using Three Spine Stickleback (*Gasterosteus aculeatus*), Report EPS 1/RM/10 Second Edition December 2017

Method Summary

Follows the Rainbow Trout and Stickleback Acute Lethality test methods but uses sea water acclimated salmon. The intent of this procedure is to outline a bioassay method for use on those effluents and waters which have a salinity >10 parts per thousand (ppt.). Salinities >10 ppt. require the use of a suitably acclimated salmonid; for this procedure, Coho (*Oncorhynchus kitsutch*) and Chinook (*O. tshawystcha*) salmon are the species of choice. It is often difficult to obtain Coho or Chinook salmon for testing. These fish are often only available from hatcheries from January to June. Alternative test species of fish may be used to assess the acute toxicity to marine fish. Topsmelt, *Atherinops affinis*; Inland Silverside, *Menidia beryllina* and Stickleback, *Gasterosteus aculeatus* can all be used for 96 hour acute toxicity tests.

It is assumed that the lab has a certain degree of familiarity with aquatic toxicity testing. Explicit instructions on every detail that might be required are not provided here. The lab is advised that the procedures or conducting of the bioassay and the care of the fish stocks (i.e. holding and acclimating) will follow the methods as described in the Environment Canada document; Biological Test Method: Acute Lethality Test Using Threespine Stickleback, Report EPS 1/RM/10, except where noted in this document. Labs are also strongly advised to review the 1/RM/9 and 1/RM/13 Rainbow Trout test methods as well.

Applications

For samples with a salinity >10 ppt. Industrial effluents (pulp mill and mining) discharged into marine water. Pure chemicals.

Sample Considerations

The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand, extremes in pH, extreme concentrations of suspended solids. Control/dilution water exhibiting extremes of salinity or containing suspended solids, toxic chemicals or metals, may cause problems. Precautions must be taken to ensure proper handling of test organisms including proper acclimation, freedom from disease and previous prophylactic treatment.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Expel all air pockets. If

warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

For an LC50 test, a minimum of 2–4, 20 L plastic cube-shaped containers or carboys are required For a pass/fail single concentration test 1–2, 20 L container(s) or carboy(s) are required.

Note: This guidance is provided for fish weighing up to 2.0 g. If larger fish are used in the tests, a larger sample volume will be required. This information can be communicated from the laboratory involved with testing.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 5 days from collection.

Endpoints

% Mortality, 96hr LC50

Quality Control

a) Reference toxicant warning chart data on test fish.
b) Negative control.
c) Routine chemistry of holding and dilution water.
d) Stock history of test fish.

Acceptability Criteria

Greater than 10% mortality or exhibition of atypical/stressed behaviour of the control fish renders the test invalid. The normal biological variation among individual fish also limits precision in a bioassay. Specific toxicity results are accurate only for the exact test parameters used, such as dilution water salinity, and fish health conditions.

Interpretation

For the pass/fail single concentration and LC50 tests an effluent sample is considered to have failed if at 100% concentration, more than 50% of the test fish die after 96 hours of exposure. For the LC20 test an effluent sample is considered to have failed if at 100% concentration, more than 20% of the test fish die after 96 hours of exposure.

Tests Organisms

Oncorhynchus kitsutch and *O. tshawyscha* may be used as the test species. Underyearling life stages are be used as test fish. The average wet weight of the test fish should be between 1 to 2.5 grams, but may be larger if loading density is maintained at ≤ 0.5 g/L. The length of the largest fish should not be more than twice that of the smallest in the same test. Mean fork length and wet weights must be measured routinely for a representative sample of fish, plus calculation of condition factor, to ensure adequate loading rates and uniformity of size in tests. Fish can be acclimated to various salinities without difficulty and be suitable for testing in less than three weeks.

All fish used in the test must be derived from the same population and source, and should be free of known diseases. Fish may be cultures or obtained from hatcheries or fish farms. Procurement and shipment of fish must be approved by the Federal-Provincial transplant committee.

Fish should be held within the temperature range compatible with good fish health (10±2°C) and ideally for at least two weeks prior to use and within ±5 ppt. salinity of that for the control/dilution water to be used in the bioassay.

Control/Dilution Water

As specified and/or depending on intent; laboratory seawater or “upstream” receiving water for monitoring and compliance; if effluent has to be salt water adjusted using a marine salt mix conduct concurrent control using suitable freshwater and adjust salinity using same marine salt mix, also conduct concurrently a second control with the salt water in which fish have been held/reared. If receiving water is used as the dilution and control water, an additional control is required using the uncontaminated water supply to which the fish were previously acclimated.

Salinity:
Normally not adjusted; if sample is essentially fresh water and it is desired to determine the toxicity at a specific salinity, use dry ocean salts or hyper brine solution (HSB*) to adjust.

Control/Dilution Water:
As specified and/or depending on intent; utilize laboratory seawater or upstream water for monitoring and compliance; if effluent has to be salt water adjusted using a marine salt mix conduct concurrent control using suitable freshwater and adjust salinity using same marine salt mix, also conduct concurrently a second control with saltwater fish have been held/reared in the same marine salt mix.

Source:
Depending on laboratory's capabilities fish may be held and acclimated in either an uncontaminated supply of natural seawater or "artificial" seawater (marine salt mix). The seawater used must have previously been demonstrated to consistently and reliably support good, survival, health, and growth of fish. The water supply should be monitored and assess routinely as required to document its quality.

Artificial seawater is prepared by adding dry ocean salts to a suitable freshwater source in quantities sufficient to reach the salinity of interest. Use only fresh sea salt mix to ensure complete dissolving of the salt mix. Commercial suppliers of dry ocean salts can be obtained from any local pet store that deals with aquarium supplies.

Ocean salts may also be added to natural seawater to raise the salinity of natural seawater. Saltwater may also be frozen and the initial melt water, hypersaline brine (HSB*) solution used to adjust salinity.

Sources of water used for preparing artificial seawater may be deionized water or distilled; or an uncontaminated supply of natural surface water or groundwater; or dechlorinated city tap water.

Salinities must be measured with recognized methods or instrumentation.

References

a) Blackburn J. and Clarke, W.C.; Revised Procedure for the 24 hour Seawater Challenge Test To Measure Seawater Adaptability of Juvenile Salmonids. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1515. January 1987. DFO Fisheries Research Branch, Pacific Biological Station, Nanaimo.

b) Environment Canada, Biological Test Method: Acute Lethality Test Using Three Spine Stickleback (*Gasterosteus aculeatus*), Report EPS 1/RM/10 Second Edition December 2017.

c) Environment Canada, Biological Test Method: Acute Lethality Test Using Rainbow Trout, Report EPS 1/RM/9 July 1990 (with May 1996 and May 2007 amendments).

b) Environment Canada, Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout, EPS 1/RM/13 Second Edition December 2000 (with May 2007 and February 2016 amendments)

e) Environment Canada, Revised Procedures For Adjusting Salinity Of Effluent Samples For Marine Sublethal Toxicity Testing Conducted Under Environmental Effects Monitoring (EEM) Programs, Method Development and Applications Section, Environmental Technology Centre, December 2001.*

Revision History

May 1997:	Method developed by PESC on behalf of the ministry.
	Method distributed to bioassay laboratories.
December 31, 2000:	Method inserted into main Laboratory Manual with minor reformatting and editing. EMS codes added.
May 03, 2018	BCELTAC Microbiology Toxicology Subcommittee

Daphnia, (*Daphnia magna*) 48 Hour Acute Lethality Test (Freshwater)

Parameter

Daphnia Acute Lethality

Test Method

Daphnia Acute Lethality Test (≤ 4 ppt. salinity)

EMS Code

Species	Test	Units	EMS Code
Daphnia magna	48hrLC50	%(v/v)	**DMGC X296**
	Pass/Fail	% mortality	

EC Test Method

Biological Test Method: Acute Lethality Test Using *Daphnia* spp., Report EPS 1/RM/11 July 1990 (including May 1996 and May 2007 amendments).

Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*, EPS 1/RM/14 Second Edition December 2000 (with February 2016 amendments).

Method Summary

Daphnia neonates (<24 hours old) are exposed in single or multiple concentration(s) of the sample and mortality is recorded over a period of 48 hours at 20±2°C. The pass/fail single concentration test uses undiluted material at 100% concentration and determines the percent mortality after 48 hours. The LC50 and LC20 tests use multiple concentrations of material diluted in laboratory water and determines the concentration of that is lethal to 50% and 20% of the test daphnids respectively.

Applications

Industrial effluents (pulp mill and mining). Landfill and woodwaste leachates. Municipal wastewater. Agricultural runoff. Elutriates. Pure chemicals. Samples must have a salinity ≤4 ppt.

Sample Considerations

The following sample properties may affect the test results: instability, extreme volatility, excessive oxygen demand, extreme pH, the presence of suspended solids. Precautions must be taken to ensure proper handling of the test organisms, including proper diet, age and lighting. Control or dilution water containing suspended solids, metals or toxic chemicals or exhibiting extremes of hardness may cause problems.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Fill with no head space. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

For an LC50 test two 1 L plastic containers are required. For a pass/fail single concentration test a 1 L plastic container is required.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 5 days from collection.

Endpoints

% Mortality, 48hrLC50, 48hrLC20

Quality Control

a) Reference toxicant warning chart data on test daphnids.
b) Negative control.
c) Routine chemistry of holding and dilution water.
d) Culture history of test organisms.

Acceptability Criteria

Greater than 10% mortality or exhibition of atypical/stressed behaviour of the control daphnids (combined replicate data), or if >2 daphnids in any single vessel exhibit either of those responses, renders the test invalid. The normal biological variation among individual daphnia also limits precision in a bioassay. Specific toxicity results are accurate only for the exact test parameters used, such as dilution, water hardness, and daphnia health conditions.

Interpretation

For the pass/fail single concentration and LC50 tests an effluent sample is considered to have failed if at 100% concentration, more than 50% of the test daphnids die after 48 hours of exposure. For the LC20 test an effluent sample is considered to have failed if at 100% concentration, more than 20% of the test daphnids die after 48 hours of exposure.

References

a) Environment Canada, Biological Test Method: Acute Lethality Test Using *Daphnia* spp., Report EPS 1/RM/11 July 1990 (with May 1996 and May 2007 amendments).

b) Environment Canada, Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*, EPS 1/RM/14 Second Edition December 2000 (with February 2016 amendments).

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
December 31, 2000:	SEAM codes replaced by EMS codes. References updated. Units added.
June 23, 2017:	BCELTAC Microbiology Toxicology Subcommittee

Photobacteria Bioassay (*Vibrio fischeri*) (Microtox) 5- and 15-min Acute Liquid-Phase Test (Fresh and Marine water)

Parameter

Inhibition of bacterial luminescence

Test Method

Acute Liquid-Phase Microtox™ (fresh and marine water)

EMS Code

Species	Test	Units	EMS Code
Vibrio fischeri	*5 min. IC50	%(v/v)	**0457 X393**
Vibrio fischeri	*15 min. IC50	%(v/v)	**0458 X393**

*with Microtox™ Model 500 analyzer

EC Test Method

Environment Canada, Biological Test Method: Toxicity Test Using Luminescent Bacteria (*Photobacterium phosphoreum*), Report EPS 1/RM/24 November 1992.

Method Summary

The Microtox™ test organism is a marine luminescent bacteria (*Vibrio fischeri*). The Microtox™ analyzer measures the light output before and after the bacteria are exposed to a dilution series of concentrations of an effluent sample at 15±1°C. The degree of light loss (an indication of metabolic inhibition) indicates the degree of toxicity of the sample. The 5- and 15-minute IC50 is the concentration of an effluent sample diluted in 2%NaCl (diluent) that is calculated to cause a 50% inhibition in light emission from the Microtox™ bacteria over an exposure period of 5 and 15 minutes.

Applications

Industrial effluents (pulp mill and mining). Landfill and woodwaste leachates. Municipal wastewater. Agricultural runoff. Elutriates. Pure chemicals. Samples can have salinity ranging from fresh to salt water.

Sample Considerations

The following sample properties may affect the test results: instability, extreme volatility, excessive oxygen demand, extreme pH, the presence of suspended solids.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Fill with no head space. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

A 125 mL sample collected in a plastic container is required.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 3 days from collection.

Endpoints

5min IC50 and 15-min IC50

Quality Control

a) Reference toxicant warning chart data on Microtox™ bacteria.
b) Negative control.
c) Routine chemistry of dilution water.
d) Certificate of Analysis for lot history of Microtox™ bacteria (includes quality control testing).

Acceptability Criteria

Valid numerical estimate of IC50 should be based on concentrations showing light inhibition both greater and less than the IC50 value. The normal biological variation among Microtox™ bacterial lots also limits precision in a test. Specific toxicity results are accurate only for the exact test parameters used, such as dilution, water hardness, and Microtox™ bacteria lot sensitivity.

Interpretation

An effluent sample with an IC50 of greater than 100% concentration is determined to be not acutely toxic to the Microtox™ bacteria. An effluent sample with an IC50 of less than 100% concentration is determined to be

acutely toxic to the Microtox® bacteria. This bioassay may be useful to predict effects to micro-organisms. In this way, the Microtox™ test can be used for the rapid toxicity screening of numerous small volume samples.

References

a) Environment Canada, Biological Test Method: Toxicity Test Using Luminescent Bacteria (*Vibrio fischeri*), Report EPS 1/RM/24 November 1992.

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
December 31, 2000:	SEAM codes replaced by EMS codes. References updated by Graham van Aggelen. Units added. Note change in species name. Minor editing.
June 23, 2017	BCELTAC Microbiology Toxicology Subcommittee

Sublethal Toxicity Test Methods, Freshwater

Salmonid Early Life Stage Sublethal Test (Freshwater)

Parameter	Viability of salmonid alevin, embryos and/or fry.
Test Method	Sublethal early life stage toxicity test, embryo, embryo/alevin and embryo/alevin fry options.
EMS Code	**SEAL X391**
EC Test Method	Biological Test Method: Toxicity Tests Using Early Life Stages of Salmonid Fish (Rainbow Trout) EPS1/RM/28 Second Edition: July 1998
Method Summary	Sublethal toxicity tests are conducted with rainbow trout eggs and milt to examine effects on embryos, alevins and swim-up fry. Three test options are available: the embryo (E) test, the embryo/alevin (EA) test, and the embryo/alevin/fry (EAF) test. Each test starts after fertilization at the onset of embryo development. The E test is terminated 7d after fertilization. The EA test is terminated 7 days after half of the alevins have hatched in the control. The EAF test ends after approximately 30 days of feeding swim-up fry.
Applications	Industrial effluents. Landfill and woodwaste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.
Sample Considerations	Effluent sample volumes can be significantly reduced using the Canaria et al. 1999 method. Test vessel volumes are reduced from 6 L to 2 L. This significantly reduces the volume of sample that must be collected and shipped to the laboratory for testing. For example, The Canaria method requires 80 L vs. 180 L for the standard Environment Canada method. The Canaria method may also improve test performance resulting in reduced incidences of tests failing to meet the validity criteria. Rainbow trout eggs are only available in season, spring and fall and transplant approval may be required. Care must be taken to ensure that eggs used for the tests are viable and are not diseased. Control or dilution water used must be at the correct temperature and have a hardness similar to the effluent being tested.
Sample Handling and Preservation	No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.
Sample Volume	Sample volumes may range from 80–160 L or more depending on the laboratory and duration of the test. The testing laboratory should be consulted prior to sample collection to ensure that adequate sample volumes are collected.
Stability	Store in dark at 4±2°C until ready for testing. M.H.T. = 5 days from collection

Endpoints

E test: EC50 and/or EC25 for nonviable embryos
EA test: EC50 and/or EC25 for nonviable alevins (failure to reach alevin stage); narrative statements on delayed hatching and deformed alevins

EAF test: EC50 and/or EC25 for nonviable individuals at swim-up (failure to survive at any stage up to time of early swim-up); LC50 for swim-up fry; IC25 for average dry weight of surviving swim-up fry at test end; narrative statements on deformed alevins, delayed swim-up and abnormal behavior of fry.

Quality Control

a) Reference toxicants.
b) Genetic history and disease treatment of egg stock.
c) Routine chemistry of holding and dilution water.

Acceptability Criteria

The test is invalid if any of the following occurs**:**

E test: >30% of controls nonviable at end of test

EA test: >35% of controls nonviable at end of test

EAF test: >40% of controls nonviable at time of 50% swim-up survivors

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) Environment Canada, Biological Test Method: Toxicity Test Using Early Life Stages of Salmonids (Rainbow Trout). Report EPS 1/RM/28. Second Edition. July 1998.

b) Canaria, E.C., J.R. Elphick, and H.C. Bailey. 1999. A Simplified Procedure for Conducting Small Scale Short-Term Embryo Toxicity Tests with Salmonids. Env. Toxicol. 14, 301–307.

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
December 31, 2000:	SEAM codes replaced by EMS codes. Out of print reference deleted. References updated. Units added.
April 2017	BCELTAC Microbiology Toxicology Subcommittee

Ceriodaphnia dubia Three Brood Survival and Reproduction Test (Freshwater)

Parameter *Ceriodaphnia* Survival and Reproduction

Test Method Three brood survival and reproduction test

EMS Code

Species	Test	Units	EMS Code
Ceriodaphnia dubia	LC50, IC25	%(v/v)	
Daphnia magna	LC50, IC25	%(v/v)	

EC Test Method Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia* EPS1/RM/21 Second Edition Highlighted February 2007.

Method Summary Recommended test for examining sublethal effects to freshwater aquatic invertebrates. Examines effects on both survival and reproduction over three brood cycles. The test duration depends on the length of each brood cycle. *Ceriodaphnia dubia* typically have a brood cycle of approximately 2 days. Whereas *Daphnia magna* has a brood cycle of approximately 7 days. Therefore, the test duration for *C. dubia* is approximately 7 days and the test duration for *D. magna* is approximately 21 days. Based on the brood cycle length, the test is most commonly conducted with *Ceriodaphnia dubia*. However, there may be some instances when using *D. magna* is preferable. For example, *C. dubia* may be more sensitive to very high or low water hardness than *D. magna*.

Applications Industrial effluents. Landfill and woodwaste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.

Sample Considerations *Ceriodaphnia* are one of the most sensitive bioassay test organisms. They are cultured in moderately hard water 80–100 mg $CaCO_3$/L. This water is often prepared with 20% Perrier water to provide salts and ions that promote and maintain culture health. This test is most commonly conducted with *D. magna* for evaluating toxicity of chemical products according to OECD TG 211.

Sample Handling and Preservation No preservation required. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume Samples are typically collected in 7 separate 1 L plastic bottles so that a fresh sample can be used for each day of the test. Clients may submit separate refresh samples for each 24h period. Laboratory staff should be consulted for sample volumes prior to collection and submission of samples.

Stability Store in dark at 4±2°C until ready for testing. M.H.T. = 5 days from collection

Endpoints Mortality, LC50; Reproduction, IC25

Quality Control

a) Reference toxicants.
b) Negative Control
c) Routine chemistry of holding and dilution water.

Acceptability Criteria The test is invalid if any of the following occurs**:** mean mortality of first-generation controls is >20%; if at least 60% of controls have not produced three broods within 8 days; if an average of <15 live young produced per surviving female in the control solutions during the first three broods; if ephippia are observed in any control solutions at any time.

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) Environment Canada, Biological Test Method: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia* EPS1/RM/21 Second Edition Highlighted February 2007.

Revision History

April 2017	BCELTAC Microbiology Toxicology Subcommittee

Green Alga, *P. subcapitata* 72h Growth Inhibition Test (Freshwater)

Parameter

P. Subcapitata growth inhibition

Test Method

Growth inhibition test using a freshwater alga

EMS Code

Species	Test	Units	EMS Code
P. subcapitata	IC50, IC25	%(v/v)	

EC Test Method

Biological Test Method: Growth Inhibition Test Using a Freshwater Alga EPS1/RM/25 Second Edition March 2007

Method Summary

Exponentially growing *P. subcaptitata* are exposed in a static microplate system to various concentrations of a test substance or an effluent over several generations of the algae under defined conditions. The growth of the algae exposed to the test substance is compared to growth of a negative control over 72 hours. A test substance is considered toxic when a statistically significant concentration dependent inhibition of algal growth is observed.

Applications

Industrial effluents. Landfill and woodwaste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.

Sample Considerations

Intended for use with freshwater effluents, leachates or elutriates where the salinity is ≤10g/kg. Coloured or turbid test solutions may reduce light transmittance to alga cells during the test resulting in a reduction of growth. This would be a physical inhibition of growth rather than a chemically induced toxic effect. In this case, *Lemna minor,* duckweed, may be a more appropriate species for assessing potential effects to plants*. Lemna minor* float on the surface of the water and therefore the test is not impaired by coloured or turbid test solutions.

P. subcapitata is a freshwater green alga that can be used to represent an important trophic level in the environment. Samples that cause growth inhibition effects could reduce algal growth in the environment and cause negative effects to the food chain required to support the life of aquatic invertebrates and fish.

In contrast, some samples can cause hormesis or a stimulation of algal growth. In this instance, it may be an indication that the wastewater or effluent may produce conditions in the environment that favors algal growth. In some cases, this may result in algal blooms or eutrophication of water systems.

Sample Handling and Preservation

No preservation required. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample volume

One 1 L sample collected in a plastic bottle.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 5 days from collection

Endpoints

Reduction of cell yield IC50, IC25

Quality Control

a) Reference toxicants.
b) Negative Control
c) Routine chemistry of holding and dilution water.

Acceptability Criteria

For a valid test, each of the following must be met**:**
Homogeneity must be demonstrated for the standard control wells, among the measurements or photometric estimates of cell yield. For a valid test, the coefficient of variation, CV, must be ≤20%.

Where the CV in the standard control wells is ≥10% but ≤20%, a trend analysis (*Mann-Kendall test*, see Gilbert, 1987) must be applied to estimates of cell yield in the standard control wells and must indicate that there is no trend or gradient in algal cell concentration across the control treatment ($p>0.05$), and

the number of algal cells measured or estimated (if photometry is used) for the standard controls must have increased by a factor of >16 in 72 hours.

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation. For any test which uses a water source other than reagent water as the control/dilution water, particular attention should be given to a comparison of algal growth in the control/dilution water with that in the standard controls using reagent water. This comparison is necessary to determine whether the control/dilution water is phytotoxic. Also, controls must be compared statistically using trend analysis (Mann-Kendall test Gilbert, 1987) to detect any effect of volatiles (if present) in the sample (see Section 4.4 of EPS1/RM/25 for details).

Any enhanced growth in test solutions, relative to that in the control solutions mush be considered when interpreting the test results and reported. Enhanced growth in test samples relative to control is often referred to as hormesis. Hormesis may indicate that wastewaters may have nutrients that can promote algal growth and potentially lead to conditions of poor water quality caused by enhanced algal growth.

References

a) Environment Canada, Biological Test Method: Growth Inhibition Test Using a Freshwater Alga EPS1/RM/25 Second Edition March 2007.

Revision History

March 2018	BCELTAC Microbiology Toxicology Subcommittee

Duckweed — *Lemna minor* 7d Growth Inhibition Test (Freshwater)

Parameter

Lemna minor growth inhibition

Test Method

Aquatic macrophyte, *Lemna minor* growth inhibition test

EMS Code

Species	Test	Units	EMS Code
Lemna minor	IC50, IC25	%(v/v)	

EC Test Method

Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor* EPS1/RM/37 Second Edition January 2007

Method Summary

The growth inhibition test is conducted at 25 ± 2°C in test vessels containing ≥100 mL of test solution and two, 3-frond plants. The test may be run as a multi-concentration assay to estimate an IC50 or IC25, or with only one concentration as a regulatory or pass/fail test. This test uses >3 replicated test vessels/treatment for a single-concentration test, and >4 replicated test vessels/treatment for a multi-concentration test.

Applications

Mine effluents. Currently used as part of Metal Mining Environmental Effects Monitoring program or Metal Mining Liquid Effluent Regulations. Industrial effluents. Landfill and wood-waste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.

Sample Considerations

Intended for use with freshwater effluents, leachates or elutriates where the salinity is ≤10g/kg. This test may be a good substitute for the freshwater algae test for coloured or turbid samples where light transmission may be reduced. This can result in a reduction of algal growth which may confound toxicity determinations. The *Lemna minor* test is not affected by reductions in aqueous light transmission because the plants float on the water surface.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

Usually a single 2 L sample may be submitted in a plastic bottle. Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 5 days from collection

Endpoints

The endpoints for the test are frond number and frond dry weight at the end of a 7-day toxicity test. Endpoints are expressed as IC50 or IC25.

Quality Control

a) Reference toxicants.
b) Negative Control
c) Routine chemistry of holding and dilution water.

Acceptability Criteria

The mean number of fronds in the controls must have increased to ≥8 times the original number of fronds by the end of the 7-day test period in order for the test to be valid. In other words, the mean number of fronds in the controls must be >48 per test vessel at the end of the test for the test to be valid.

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

For any test which uses a water source other than SIS medium, modified Steinberg medium or, where appropriate, modified APHA medium as the control/dilution water, particular attention should be given to a comparison of Lemna growth in the control/dilution water with that in the standard controls using test medium (SIS, Steinberg, or APHA). This comparison is necessary to determine whether the control/dilution water is phytotoxic. Any enhanced growth in test solutions, relative to that in the control solutions, must be reported and considered when interpreting the findings (see Sections 4.5.2 and 4.5.4 in EPS1/RM/37).

Enhanced growth in test samples relative to control is often referred to as hormesis. Hormesis may indicate that wastewaters may have nutrients that can promote algal growth and potentially lead to conditions of poor water quality caused by enhanced algal growth.

References

a) Environment Canada, Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor* EPS1/RM/37 Second Edition January 2007.

Revision History

Mar h 2018	BCELTAC Microbiology Toxicology Subcommittee

Sublethal Toxicity Test Methods, Marine/Estuarine

Topsmelt 7d Growth and Survival Test (Marine Water)

Parameter

Topsmelt growth and survival

Test Method

Topsmelt 7 day growth and survival test

EMS Code

Species	Test	Units	EMS Code
Atherinops affinis	LC50, EC25	%(v/v)	

US EPA Test Method

Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA 600/R-95/136 August 1995.

Method Summary

This test is used to evaluate the chronic toxicity of effluents and receiving waters to the topsmelt, *Atherinops affinis*. Topsmelt are a relevant, west coast species. Effects are evaluated on 9 to 14 days old larvae during a 7-day static renewal exposure test. The test can be conducted as a single concentration, pass/fail test with negative control, or more commonly as a multi-concentration (minimum 5) with a negative control. Growth is evaluated by measuring the dry weight of test organisms and comparing them to controls.

Applications

Industrial effluents. Landfill and wood-waste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.

Sample Considerations

Topsmelt are marine organisms. If fresh water effluents are tested, they must be salinity adjusted using hypersaline brine, (HSB) solutions or artificial sea salts to ensure that the test salinity is matched to the salinity of the receiving water. Test samples are diluted with HSB or sea salt solutions to prepare test dilutions. Methods for preparation of brine solutions and or sea salt are documented in the EPA test method.

Sample Handling and Preservation

No preservation required. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

Typically a 40 L sample is required which can be collected in 4 separate 10 L plastic cubitainers. Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 36h from collection

Endpoints

Mortality or survival, LC50 and growth inhibition (dry weight) IC50, IC25.

Quality Control

a) Reference toxicants.
b) Negative Control
c) Routine chemistry of holding and dilution water.

Acceptability Criteria

Survival in controls ≥80%, 0.85 mg average weight of control larvae (9 day old).

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA 600/R-95/136 August 1995.

Revision History

March 2018 BCELTAC Microbiology Toxicology Subcommittee

Echinoderm (Sea Urchin and Sand Dollar) 20 min Sublethal Fertilization Test (Marine Water)

Parameter

Inhibition of echinoiderm fertilization

Analytical Method

Marine/estuarine echinoderm bioassay (> 25ppt salinity), IC50, IC25.

EMS Code

Species	Test	Units	EMS Code
Dendraster excentricus (Sand dollar) or, *Strongylocentrotus purpuratus* (Purple sea urchin)	IC50, IC25	%(v/v)	**ECHI X395**

EC Test Method

Biological Test Method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars) EPS 1/RM/27 Second Edition — February 2011.

Method Summary

Eggs and sperm are collected from the animals. Sperm is added to serial diluted concentrations of material for 10 minutes. Sperm is added to eggs, and chemically fixed after ten minutes. The percent unfertilized eggs is determined by microscopic examination. The concentration causing 50% fertilization inhibition IC50 is the test endpoint. The Puget Sound Environmental Protocol (PSEP) method requires a 20-minute exposure of sperm to effluent and a 20-minute fertilization exposure where sperm and eggs are exposed together.

Application

Industrial effluents. Landfill leachates. Municipal wastewater. Pure chemicals. Modified SWEP extracted sediments (elutriates).

Interferences and Precautions

Choice between sand dollars and sea urchins is seasonal, based on when test organisms are gravid: sea urchins in winter–spring, (January–May) and sand dollars in summer–fall (May–October). Which species is used as the test organism should be noted in the EMS comment. The single EMS code applies to both organisms.

Tests should be conducted with test organisms within the time that the echinoid species is gravid. Salinity adjustment with sea salts or hypersaline brine is required for effluents <25 ppt salinity.

Sample Handling and Preservation

Samples should be shipped in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation. No preservation required.

Sample Volume

Typically, a single 1 L plastic bottle can be used to submit the sample. Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability

Store in dark at 4±2°C. M.H.T. = 5 days.

Endpoints

Fertilization inhibition, IC25

Validity criteria

A test is invalid if the mean fertilization rate for all replicates of the control water is <60%, or ≥98%. Also, a positive and logical concentration-effect curve should have been attained for the results to be considered valid. In other words, there should be a concentration dependent response observed. If dissolved oxygen in one or more test vessels is <40% saturation, the test may be considered an invalid assessment of the toxicity of the sample being tested.

Quality Control

a) Negative control
b) Reference toxicant warning charts.
c) Routine chemistry of holding and dilution water.
d) Animals collected from “clean sites”.

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) Biological Test Method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars) EPS 1/RM/27 Second Edition — February 2011.

Revision History

March 2018 BCELTAC Microbiology Toxicology Subcommittee

Pacific Oyster, *Crassostrea gigas* and Mussel, *Mytilus* sp. Embryo Larval Development Test Method (Marine Water)

Parameter

Bivalve larval development (liquid phase)

EMS Code

Species	Test	Units	EMS Code
Crassostrea gigas (Pacific Oyster)		%(v/v)	**ECHI X395**
or,			
Mytilus sp. (Pacific Mussels)			

Note: The mussel species most commonly used in British Columbia for this test are *Mytilus edulis* and *Mytilus galloprovinciallis*. Which species is used as the test organism should be noted in the EMS comment. The single EMS code applies to both organisms.

Test Method

Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA 600/R-95/136 August 1995.

Method Summary

This method is used to examine the effects of effluent on the development of bivalve larvae over a 48h static non-renewal exposure. Adult organisms are spawned and freshly fertilized eggs (embryos) are exposed to whole effluent samples. Each test consists of a seawater control, and four replicates of at least five dilutions of test effluent.

At the end of the 48h exposure period, control organisms should be evaluated to check for complete development of the control organisms. If development is complete, the test is ended. If development is incomplete, then the test is continued until the organisms are fully developed but not past 54 hours from the test initiation.

Endpoints

Survival and normal shell development.

Applications

Effluents discharged into marine or estuarine environments.

Sample Considerations

The oysters and mussels used in this test are marine organisms. If fresh water effluents are tested, they must be salinity adjusted using hypersaline brine, (HSB) solutions or artificial sea salts to ensure that the test salinity is matched to the salinity of the receiving water. Test samples are diluted with HSB or sea salt solutions to prepare test dilutions. Methods for preparation of brine solutions and or sea salt are documented in the EPA test method.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Fill with no head space. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±3°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

Typically one Litre plastic bottle completely filled with effluent. Sample volumes must be discussed with laboratory staff.

Stability

Store in dark at 4±2°C in airtight containers until ready for testing. It is recommended that samples are tested as soon as possible after collection. M.H.T. = 36h from collection.

Validity Criteria	Control survival must be $70% for oyster embryos or $50% for mussel embryos in control vials; $90% normal shell development in surviving controls; and must achieve a %MSD of <25%
Quality Control	a) Reference toxicant warning charts. b) Routine chemistry of test and control samples. c) Test organisms must be collected from “clean sites”.
Interpretation	The IC25 value is estimated using statistical methods based on the test data.
References	a) Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA 600/R-95/136 August 1995.
Revision History	September 2018 BCELTAC Microbiology Toxicology Subcommittee

Giant kelp, *Macrocystis pyrifera* 48hr Sublethal Toxicity Test (Marine Water)

Parameter Pacific kelp bioassay

Test Method

Species	Test	Units	EMS Code
Macrocystis pyrifera	IC50, IC25	%(v/v)	

EMS Code

EPA Test Method Test Method: Giant Kelp, *Macrocystis pyrifera*, Germination and Germ-Tube Length Test Method 1009.0 in: Short-term Methods For Estimating The Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (First Edition) EPA/600/R-95-136 August 1995.

Method Summary The chronic toxicity of effluents or receiving waters is evaluated using kelp zoospores and embryonic gametophytes of a Pacific giant kelp species, *Macrocystis pyrifera*. The test is conducted for 48h under static exposure conditions. The test can be run as a single concentration, pass/fail test with a control or with multiple concentrations, (minimum of 5) with a control. Kelp fronds are placed in dry conditions to mimic low tide and then are placed in beakers in seawater to mimic high tide and stimulate production of zoospores.

Applications Industrial effluents. Landfill and woodwaste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.

Sample Considerations Salinity adjustment with sea salts or hypersaline brine is required for effluents <25 ppt salinity.

Sample Handling and Preservation No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume Typically, a single 1 L plastic bottle can be used to submit the sample. Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability Store in dark at 4±2°C until ready for testing. M.H.T. = 36h from collection

Endpoints There are two test endpoints, percent germination success of gametophyte spores and length of embryonic gametophyte tubes (µm). Tube length is measured microscopically with an ocular micrometer or by measuring photographs of test organisms using an appropriate software program. **An inhibitory concentration, e.g. IC25 value can be estimated for each endpoint.**

Quality Control

a) Reference toxicants.
b) Negative Control
c) Routine chemistry of holding and dilution water.

Acceptability Criteria For tests to be considered acceptable, the following requirements must be met**:**
Mean control germination must be ≥70% in the controls.
Mean germination-tube length in the controls must be ≥10µm in the controls.

The minimum significant difference (%MSD) is <20% relative to the control for both germination and germ-tube length in the reference toxicant test.

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) Test Method: Giant Kelp, *Macrocystis pyrifera*, Germination and Germ-Tube Length Test Method 1009.0 in: Short-term Methods For Estimating The Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (First Edition) EPA/600/R-95-136 August 1995.

Revision History

March 2018	BCELTAC Microbiology Toxicology Subcommittee

Red algae, *Champia parvula* 7d Sublethal Reproduction Test (Marine Water)

Parameter

Red Microalga, *Champia parvula*, Sexual Reproduction Inhibition

Test Method

Species	Test	Units	EMS Code
Champia parvula	LC50, IC25	%(v/v)	

EMS Code

Test Method

EPA-821-R-02-014 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, October 2002. Red Microalga, *Champia parvula*, Sexual Reproduction Test Method 1009.0

Method Summary

This test measures the effects of toxic substances in effluents and receiving water on the sexual reproduction of the marine red macroalga, *Champia parvul*a. The method consists of exposing male and female plants to test substances for two days, followed by a 5–7 day recovery period in control medium, during which the cystocarps mature. The test is very challenging and can be confounded if male and female plants are not isolated in cultures.

Applications

Environmental Effects Monitoring Program, Industrial effluents. Landfill and woodwaste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.

Sample Considerations

Salinity adjustment with sea salts or hypersaline brine is required for effluents <25 ppt salinity.

Sample Handling and Preservation

No preservation required. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

Typically, a single 1 L plastic bottle can be used to submit the sample. Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 36h from collection

Endpoints

LC50 mortality, IC25 Reproduction

Quality Control

a) Reference toxicants
b) Negative Control
c) Routine chemistry of holding and dilution water.
d) Test organism culture records.

Acceptability Criteria

The test is acceptable if (1) control survival equals or exceeds 80% and (2) control plants average 10 or more cystocarps per plant. If plants fragment in the controls or lower exposure concentrations, it may be an indication that they are under stress.

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) EPA-821-R-02-014 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, October 2002. Red Microalga, *Champia parvula*, Sexual Reproduction Test Method 1009.0

Revision History

March 2018 BCELTAC Microbiology Toxicology Subcommittee

Mysid Shrimp, *Americamysis bahia* 7d Survival and Growth Test (Marine Water)

Parameter Mysid shrimp, *Americamysis bahia*, Survival and Growth

Test Method

Species	Test	Units	EMS Code
Americamysis bahia	LC50, IC25	%(v/v)	

EMS Code

Test Method EPA-821-R-02-014 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, October 2002. Test Method: Mysid, *Mysidopsis bahia*, Survival, Growth, and Fecundity Test Method 1007.0.

Method Summary This test measures the effects of toxic substances in effluents and receiving water on the growth and survival of the mysid shrimp, *Americamysis bahia*, (formerly called *Mysidopsis bahia*). The method consists of exposing test organisms to wastewaters or other test substances over a 7-day period with no test solution renewal.

It is recommended to conduct the test as a growth and survival test without examining the fecundity endpoint described in the USEPA test method because mysid shrimp are cannibalistic and therefore may eat they young which can confound the fecundity endpoint. Many laboratories have difficulty conducting the test so that a reliable fecundity endpoint is obtained.

The test can also be shortened to an acute test, 48h or 96h. In this case, only acute lethality is examined following static exposure. The test is normally conducted with a minimum of five concentrations and a seawater control.

Applications Environmental effects monitoring, industrial effluents, landfill and wood-waste leachates. Municipal wastewater/storm water. Agricultural runoff. Pure chemicals.

Sample Considerations The salinity range of test water must be 20–30 ppt. Salinity adjustment with sea salts or hypersaline brine is required for effluents <20 ppt salinity.

Sample Handling and Preservation No preservation required. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume A volume of 30 L is often requested. Three clean, new plastic 10 L square shaped carboys can be used to submit the sample. Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability Store in dark at 4±2°C until ready for testing. M.H.T. = 36h from collection

Endpoints LC50 mortality, IC25 Growth

Quality Control

a) Reference toxicants
b) Negative Control
c) Routine chemistry of holding and dilution water.
d) Test organism culture records.

Acceptability Criteria Survival: **≥**80%, growth: average dry weight 0.20 mg or greater in controls (required).

Interpretation

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) EPA-821-R-02-014 Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms Third Edition, October 2002. Test Method: Mysid, *Mysidopsis bahia*, Survival, Growth, and Fecundity Test Method 1007.0.

Revision History

September 2018 BCELTAC Microbiology Toxicology Subcommittee

Sediment Toxicity Test Methods, Freshwater Sediment

Amphipod (*Hyalella azteca*) 14d Acute Toxicity Test (Freshwater Sediment)

Parameter

Amphipod acute toxicity

Analytical Method

Freshwater sediment bioassay, 14 day survival and growth toxicology

EMS Code

Species	Test	Units	EMS Code
Hyalella azteca	14-day survival, growth	%(v/v)	HAGC X392

Test Method

Environment Canada Biological Test Method: Test for Survival and Growth in Sediment Using the Freshwater Amphipod *Hyalella azteca.* EPS 1/RM/33 Second Edition, January 2013.

Method Summary

The method has been significantly updated by Environment Canada since the original edition published in December 1997. Updates include options for sediment-water ratio, age of test organisms, use of replicates, overlying water renewal, food types and feeding rates, light intensity for culturing and statistical analysis of data. Normally conducted at 23 ± 1°C in glass vessels containing 100 mL of sediment and 175 mL overlying water. An option for a 1:4 sediment to water ratio is included. The test is conducted as a static renewal exposure with a minimum of 5 replicates per treatment.

Applications

Freshwater sediment, ≤15‰ salinity, soil and sludge. Pure chemicals.

Sample considerations

The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand, presence of sulfides and/or ammonia, pH levels outside of organism tolerance. Field collected sediments may contain indigenous organisms including predators, and the same or closely related species. Control/ dilution water exhibiting extremes in hardness, or suspended materials, or variable temperature may cause problems. Test organisms must be acclimated to dilution water and the correct life stage must be used.

Sample Handling and Preservation

Upon collection sediment samples should be cooled to between 1–7°C prior to shipping in coolers with ice to the laboratory. Samples should be stored at 4±3°C in the dark and testing should be started within two weeks of collection. Samples may be stored for a maximum of six weeks prior to testing. Samples must not be frozen. Any air or headspace in the storage container should be minimized and purged with nitrogen gas.

Sample Volume

Sediments should be collected in five separate 1 L buckets according to the latest revision of the Environment Canada test method (EPS 1/RM/33 Second Edition, January 2013). This allows for incorporation of five field replicates into the laboratory test. However, 2 L sediment samples may be collected and laboratory replicates would be prepared from a single sample.

Stability

No preservation required. Store in dark at 4°C. M.H.T. = 2 weeks to 6 weeks from collection.

Endpoints

Survival/mortality LC50, growth IC25 based on dry weight of test organisms.

Acceptability Criteria

Minimum 80% control survival required for valid testing. The test is invalid if the average dry weight for the replicate control groups is <0.1 mg per individual amphipod surviving at the end of the test. Reference toxicant tests must be conducted in conjunction with the tests. Warning charts are required.

Quality Control

a) Reference toxicant warning charts.
b) Routine chemistry of holding and dilution water.
c) Test organisms must be collected from “clean sites”.
d) Controls with dilution water only and with “clean control sediment”.
e) Reference sediment samples should be tested for comparison to test samples.

Interpretation

Sediment test sample results should compared to those of a field collected reference sediment sample to examine potential effects to test organisms. Statistically significant reductions in survival or growth in the test sample(s) compared to the reference site may indicate the presence of toxic substances. The negative control consisting of laboratory sand is used to evaluate the performance and health of the test organisms.

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) Environment Canada Biological Test Method: Test for Survival and Growth in Sediment Using the Freshwater Amphipod *Hyalella azteca.* EPS 1/RM/33 Second Edition, January 2013.

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
December 31, 2000:	SEAM codes replaced by EMS codes. References updated. One new reference added Units added.
March 2018	BCELTAC Microbiology Toxicology Subcommittee

Chironomus 10d Survival and Growth Test (Freshwater Sediment)

Parameter

Survival and growth of *Chironomus* spp.

EMS Code

Species	Test	Units	EMS Code
Chrionomus dilutus	10 day survival, growth	%(v/v)	

Test Method

Biological Test Method: Test for Survival and Growth in Sediment Using Larvae of Freshwater Midges (*Chironomus tentans* or *Chironumus riparius*) EPS 1/RM/32 — December 1997.

Method Summary

This 10-day freshwater sediment toxicity test is conducted at 23±1°C in glass test vessels containing 100 mL of sediment and 175 mL of overlying water. A minimum of 5 test chambers each containing 10 organisms are normally used for test replicates. Note that *C. tentans* has been renamed *C. dilutus*.

Applications

Freshwater sediment, ≤15‰ salinity, soil and sludge. Pure chemicals

Sample Considerations

The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand, presence of sulfides and/or ammonia, pH levels outside of organism tolerance. Field collected sediments may contain indigenous organisms including predators, and the same or closely related species. Control/ dilution water exhibiting extremes in hardness, or suspended materials, or variable temperature may cause problems. Test organisms must be acclimated to dilution water and the correct life stage must be used.

Sample Handling and Preservation

Upon collection, sediment samples should be cooled to between 1–7°C prior to shipping in coolers with ice to the laboratory. Samples should be stored at 4±2°C in the dark and testing should be started within two weeks of collection. Samples may be stored for a maximum of six weeks prior to testing. Samples must not be frozen. Any air or headspace in the storage container should be minimized and purged with nitrogen gas.

Sample Volume

Sediments should be collected in five separate 1 L buckets according to the latest revision of the Environment Canada test method (EPS 1/RM/33 Second Edition, January 2013). This allows for incorporation of five field replicates into the laboratory test. However, 2 L sediment samples may be collected and laboratory replicates would be prepared from a single sample.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 2 weeks to 6 weeks from collection.

Endpoints

Survival/mortality LC50, growth IC25 dry weight.

Quality Control

a) Reference toxicant warning charts.
b) Routine chemistry of holding and dilution water.
c) Test organisms must be collected from “clean sites”.
d) Controls with dilution water only and with “clean control sediment”.
e) Reference sediment samples should be tested for comparison to test samples.

Acceptability Criteria

The test is invalid if any of the following occurs**:** survival in control sediment is <70%. If the mean dry weight of surviving control organisms is <0.6 mg per individual *C. dilutus* or <0.5 mg per individual *C. riparius*.

Interpretation

Sediment test sample results should compared to those of a field collected reference sediment sample to examine potential effects to test organisms. Statistically significant reductions in survival or growth in the test sample(s) compared to the reference site may indicate the presence of toxic substances. The negative control consisting of laboratory sand is used to evaluate the performance and health of the test organisms.

There are no regulatory pass/fail criteria specified by Environment Canada for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) Environment Canada, Biological Test Method: Test for Survival and Growth in Sediment Using Larvae of Freshwater Midges (*Chironomus tentans* or *Chironumus riparius*) EPS 1/RM/32 — December 1997.

Revision History

March, 2018	BCELTAC Microbiology Toxicology Subcommittee

Oligochaete Worm, Lumbriculus, 28d Bioaccumulation Test (Freshwater Sediment)

Parameter	Freshwater sediment bioaccumulation test
Test Method	EPA 600/R-94/064 Test Method 100.3: Lumbriculus variegatus Bioaccumulation Test for Sediments In: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, Second Edition, March 2000.
EMS Code	
Method Summary	Sediment is homogenized and placed into test chambers in equal amounts, and overlying water is added one day prior to test initiation. Water is then renewed prior to introduction of organisms. Oligochaetes are isolated prior to test initiation and mean group weight is measured on a subset of at least 100 organisms. Healthy organisms are then introduced to test chambers. All chambers are then checked daily and observations made to assess test organism behavior. At end of 28-day period, sediment is then sieved through a fine mesh screen. Wet weight is then determined of the survivors before they are put into a clean beaker of overlying (renewal) water for gut purging for a period of time not to exceed 24h. **The Ontario Ministry of the Environment and Climate Change has published a variation of this freshwater sediment bioaccumulation test involving an invertebrate species, the mayfly nymph *Hexagenia spp*. in addition to *Lumbriculus*, and one fish species, the fathead minnow *Pimephales promelas*. These three organisms were chosen to reflect differences in taxa, trophic level, and bioaccumulation potential (Van Geest and Watson-Leung, 2016). The test is conducted for 28d under static conditions.**
Applications	Freshwater sediment, ≤15‰ salinity, soil and sludge. Pure chemicals
Sample Considerations	The bioaccumulation test is not designed to evaluate toxicity but to evaluate bioaccumulation potential of contaminants of concern. Therefore, this test will not be effective for sediments with high acute toxicity because test organisms may not survive over the duration of the test. The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand, ammonia and sulphide. Field collected sediments may contain indigenous organisms including predators, and the same or closely related species. Test sediments must be sieved prior to testing to remove indigenous organisms. Control/dilution water should have a salinity of 25–30 ppt. Test organisms: correct species must be acclimated to dilution water. Test organisms must be obtained from clean reference areas.
Sample Handling and Preservation	No preservation required. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.
Sample Volume	The sample volume may vary depending on the testing laboratory. Sample volumes may range from 8–12L. Sample volumes and frequency of material replacement must be discussed with laboratory staff.
Stability	Store in dark at 4±2°C until ready for testing. M.H.T. = 2 weeks to less than 8 weeks from collection.

Endpoints

Bioaccumulation, a biota sediment accumulation factor (BSAF) may be calculated based on the concentrations determined in the test organisms and sediments obtained from the test.

Quality Control

a) Reference toxicant warning charts.
b) Routine chemistry of holding and dilution water.
c) Test organisms must be collected from “clean sites” and cultured in laboratory.
d) Controls with dilution water only and with “clean control sediment”.
e) Reference sediment samples should be tested for comparison to test samples.

Acceptability Criteria

It is recommended that the following performance criteria be met:

1. Numbers of *L. variegatus* in a 4-day toxicity screening test should not be significantly reduced in the test sediment relative to the control sediment.

2. Test organisms should burrow into test sediment. Avoidance of test sediment by *L. variegatus* may decrease bioaccumulation.

3. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

B. Performance-based criteria for culturing *L. variegatus* include the following:

1. Laboratories should perform 96-h water-only reference toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.

2. Laboratories should monitor the frequency with which the population is doubling in the culture (number of organisms) and record this information using control charts (doubling rate would need to be estimated on a subset of animals from a mass culture). Records should also be kept on the frequency of restarting cultures. If static cultures are used, it may be desirable to measure water quality more frequently.

3. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures. Lipid content may also be used to normalize bioaccumulation estimates.

Interpretation

The concentrations of contaminants of concern in the test organisms should be evaluated to see if there is a statistically significant increase in concentration over duration of the test. The BSAF can indicate the potential for the contaminant(s) to bioaccumulate in the food web (trophic transfer). Specifically, the test can be used to evaluate the bioaccumulative potential of contaminants in sediment dwelling worms. The negative control consisting of laboratory sand is used to evaluate the performance and health of the test organisms.

There are no regulatory pass/fail criteria specified for this test. Test results can be used to inform if sublethal toxicity may be of concern if adverse effects are observed to test organisms following exposure to the test sample. Pass/fail criteria may be specified in regulatory documentation.

References

a) EPA 600/R-94/064 Test Method 100.3: *Lumbriculus variegatus* Bioaccumulation Test for Sediments In: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, Second Edition, March 2000.

b) Van Geest J and Watson-Leung T. Bioaccumulation of sediment-associated contaminants in freshwater organisms. Aquatic Toxicology Unit of the Laboratory Services Branch, Ontario Ministry of the Environment and Climate Change, Etobicoke, ON December 2016.

Revision History

April 2017	BCELTAC Microbiology Toxicology Subcommittee

Solid-Phase Microtox™ 10 min Acute Toxicity Test (Freshwater or Marine Sediment)

Parameter

Bacterial Luminescent Inhibition

Analytical Method

Solid-Phase Microtox™ (fresh and marine sediments)

EMS Code

Species	Test	Units	EMS Code
Vibrio fischeri	*10 min. IC50	mg/L	0457 X394

*with Microtox™ Model 500 analyzer

EC Test Method

Environment Canada, Biological Test Method: Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test, Report EPS 1/RM/42 — April 2002.

Method Summary

The Microtox™ test organism is a marine luminescent bacteria (*Vibrio fischeri*). The Microtox™ analyzer measures the light output after the bacteria are exposed to a dilution series of concentrations of a sediment sample, after a 20-minute incubation followed by a 10-minute stabilization of the filtrate at at 15±1°C. The degree of light loss (an indication of metabolic inhibition) indicates the degree of toxicity of the sample. The 10-minute IC50 is the concentration of a sediment sample diluted in 3.5% NaCl (diluent) that is calculated to cause a 50% inhibition in light emission from the Microtox™ bacteria over the filtrate stabilization period of 10 minutes.

Applications

Estuarine, fresh and marine water sediments. Terrestrial soils (i.e., landfills, contaminated soils, sludges, etc.).

Sample Considerations

To obtain a well-matched “clean” reference sample, each must have the similar grain or particle size, composition, and moisture content. Sediments can contain a high level of natural toxicity (pore water ammonia and/or hydrogen sulphide) in areas that have high organic enrichment from natural sources (i.e. abundance of vegetable and animal life). “Clean” reference (negative control) sediment should be collected from each geographical sediment location. Each sample must be characterized by analyzing subsamples for particle size distribution (percent coarse = > 1.0 mm, percent sand = > 0.063 > 1.0 mm, percent fines < 0.063 mm), percent moisture, total organic carbon and pore water salinity and pH. It is also recommended to measure pore water ammonia and hydrogen sulphide.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Fill with no head space. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

A 125 mL amber glass container is required.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = preferably 2 weeks from collection and maximum 6 weeks from collection.

Endpoints

10min IC50 (dry weight)

Quality Control

a) Reference toxicant using positive control sediment for warning chart data on Microtox™ bacteria.
b) “Clean” reference (negative control).
c) Routine chemistry of dilution water.
d) Certificate of Analysis for lot history of Microtox™ bacteria (includes quality control testing).

Acceptability Criteria

Valid numerical estimate of IC50 should be based on concentrations showing light inhibition both greater and less than the IC50 value. The coefficient of variation representing the mean light reading measured from the filtrates of the 3 control solutions must be ≤ 12%. The normal biological variation among Microtox™ bacterial lots also limits precision in a test. Specific toxicity results are accurate only for the exact test parameters used, such as dilution, water hardness, and Microtox™ bacteria lot sensitivity.

Interpretation

The solid-phase Microtox® test data interpretation interim guidelines are as follows:

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. This first interim guideline, which has been recommended and applied by Environment Canada in the past is based on the premise that all samples, independent of what the grain size profiles are, are toxic according to this biological test method if their IC50 is < 1000 mg/L.

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 a percent 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 this sediment toxicity test if, and only if, each of the following apply: (1) it’s IC50 is more than 50% lower than that determined for the sample of reference sediment or negative control sediment; and (2) the IC50s for the test sediment and the reference sediment or negative control sediment differ significantly. (For Guideline 2, “fines” refers to sediment particles which are ≤ 0.063 mm in size. Measurements of percent fines include all particles defined as silt (≤ 0.063 ≥ 0.004 mm) and clay (< 0.004 mm). The second guideline is based on the premise that samples with < 20% fines might be toxic at an IC50 ≥ 1000 mg/L, since confounding grain size effects are appreciably less in coarse-grained sediment.

The two interim guidelines for judging the toxicity of samples of test sediment using this reference method are discussed in the following paragraph (1 Environment Canada, Biological Test Method: Reference Method For Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test, Report EPS 1/RM/42 April 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 < 1000 mg/L. The second interim guideline should be applied to all samples of test sediment with < 20% fines that have an IC50s ≥ 1000 mg/L. Applying the second interim guideline to samples of sediment with < 20% fines and IC50s ≥ 1000 mg/L enables toxic coarse-grained sediments to be identified as such when their IC50 is appreciably higher than 1000 mg/L. It is recommended that the second guideline be applied to each sample of test sediment with < 20% fines, except in the instance where the IC50 is < 1000mg/L in which case the sample should be judged as toxic and the second guideline does not apply. Generally coarse sediments exhibit higher IC50s than fine sediments because large grain size makes it difficult to serial dilute accurately and less surface area is available for toxicant binding.

References

a) Environment Canada, Biological Test Method: Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test, Report EPS 1/RM/42 — April 2002.

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
December 31, 2000:	SEAM codes replaced by EMS codes. References updated by Graham van Aggelen. Units added. Note change in species name. Minor editing.
June 23, 2017	BCELTAC Microbiology Toxicology Subcommittee

Sediment Toxicity Test Methods, Marine and Estuarine Sediment

Amphipod (*Eohaustorius estuarius*) 10d Acute Toxicity Test (Marine Sediment)

Parameter

Acute toxicity (mortality)

Analytical Method

Sediment-burrowing amphipods, 10 day burrowing and survival

EMS Code

Species	Test	Units	EMS Code
Rhepoxynius abronius	10 day	%(v/v)	AMGC X396
Eohaustorius estuaries	10 day	%(v/v)	

Method Summary

175–200g of sediment is added to 750–800 mL of salt water. 20 organisms added per beaker; 5 replicates per set. Burrowing and survival are recorded after 10 days.

Applications

Estuarine and marine sediment, and sludge. Note: Sediment ranging from >90% silt- and clay-size particles to 100% sand-size particles did not reduce survival in laboratory.

Sample Considerations

Where practical and consistent with the study design and objectives, a minimum of five replicate samples (i.e. field replicates) of sediment must be taken from each discrete sampling station and depth of interest. Were practical and appropriate, sample collection must also include ≥5 samples (i.e. field replicates) from each of one or more reference stations. Reference stations should consist of sites with uncontaminated sediments having physico-chemical properties similar to that of the test sediments. For example, to obtain a well-matched “clean” reference sample, each must have the similar grain or particle size, composition, and moisture content.

Sediments can contain a high level of natural toxicity (pore water ammonia and/or hydrogen sulphide) in areas that have high organic enrichment from natural sources (i.e. abundance of vegetable and animal life). “Clean” reference (negative control) sediment should be collected from each geographical sediment location. It is also recommended to measure pore water ammonia and hydrogen sulphide. Control/dilution water should have a salinity of 25–30 ppt. Suspended material and variable temperature may cause problems. Test organisms: correct species must be acclimated to dilution water. Animals must be field collected from a “clean reference” site.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Fill with no head space. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±3°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

2 L of sediment collected in a plastic pail without headspace. Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 2 weeks to 6 weeks from collection. Freezing and longer storage might change sediment properties and should be avoided.

Validity Criteria

See Table 1 for control sediment survival requirements. Five replicates are required for each sample. Reference and control sediments required. Statistical (ANOVA) calculation should be used to determine significant difference from control sediment.

Quality Control

a) Reference toxicant warning charts.
b) Routine chemistry of test, reference, and clean sediment lab control.
c) Test organisms must be collected from “clean sites”.

Interpretation

Information for test failure criterion for the various amphipod species are presented in Table 1.

TABLE 1 TEST FAILURE CRITERION

LETHALITY TEST					
	SPECIES	*REFERENCE SEDIMENT SURVIVAL*	*MEAN TEST SEDIMENT SURVIVAL IS*	*CONTROL SEDIMENT SURVIVAL*	*CONTROL SEDIMENT (IN ABSENCE OF SUITABLE REFERENCE)*
AMPHIPOD 10-DAY ACUTE LETHALITY TEST (EPS 1/RM/35)	*Amphiporeia virginiana*	≥ 70%	*> 20% lower and significantly different than reference*	≥ 80%	*> 30% lower and significantly different than control*
	Eohostorius washintonianus	≥ 75%	*> 20% lower and significantly different than reference*	≥ 85%	*> 30% lower and significantly different than control*
	Eohostorius estuarius	≥ 80%	*> 20% lower and significantly different than reference*	≥ 90%	*> 30% lower and significantly different than control*
	Rhepoxynius abronius	≥ 80%	*> 20% lower and significantly different than reference*	≥ 90%	*> 30% lower and significantly different than control*

References

a) Environment Canada, Biological Test Method: Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods. Report EPS 1/RM/26 December 1992, Amended October 1998.

Revision History

February 14, 1994: Publication in 1994 Laboratory Manual.

December 31, 2000: SEAM codes replaced by EMS codes. Units added.

December 10, 2017 BCELTAC Microbiology Toxicology Subcommittee

March 23, 2018 BCELTAC Microbiology and Toxicology Subcommittee

Echinoderm (*Dendraster excentricus* and *Strongylocentrotus purpuratus*) Larval Development Test (Marine Sediment)

Parameter Echinoderm larval development (Solid phase)

EMS Code

Species	Test	Units	EMS Code
Dendraster excentricus (Sand dollar)		%(v/v)	ECHI X395

or,

Strongylocentrotus purpuratus (Purple sea urchin)

Note: Choice between sand dollars and sea urchins is seasonal: sea urchins in summer (the fertile period), and sand dollars in winter. Which species is used as the test organism should be noted in the EMS comment. The single EMS code applies to both organisms.

EC Test Method Reference Method for Measuring the Toxicity of Contaminated Sediment to Embryos and Larvae of Echinoids (Sea Urchins or Sand Dollars). EPS 1/RM/58. July 2014.

Method Summary Freshly fertilized eggs (embryos) are exposed to whole sediment samples. Each test consists of a control sediment, test sediment and a field collected reference sediment. The test is started within 2–4 hours of fertilization. A mean fertilization success rate of ≥90% must be achieved for the test to be initiated. Embryos (approx. 200) are transferred to all test vessels containing sediment. The test duration is species dependent, 72h for *D. excentricus* and 96h for *S. purpuratus*.

The test can be prolonged by 24 ± 1hr based on the percentage of normal larvae determined in the “water-only” controls included in the test for this purpose. If at the test end, the mean % normal larvae in the monitoring vials is <70% then the test must be extended for an additional 24 hours to ensure the test validity criteria is met.

Using a “total count” approach, all embryos and larvae recovered from each replicate must be counted and scored. Counting may be performed in-vial using an inverted microscope or using a Sedgewick-Rafter cell. For each test replicate, the number of normal larvae (prism or pluteus) and abnormal larvae are counted and documented. The percentage of normal larvae is calculated as the test endpoint.

Endpoints Percent normal larvae, the test sample is compared statistically to the reference sample.

Applications Marine and estuarine sediment samples.

Sample Considerations This test is to be used on marine and estuarine sediments with a minimum salinity of 15‰.

Where practical and consistent with the study design and objectives, a minimum of five replicate samples (i.e. field replicates) of sediment must be taken from each discrete sampling station and depth of interest. Where practical and appropriate, sample collection must also include ≥5 samples (i.e. field replicates) from each of one or more reference stations. Reference stations should consist of sites with uncontaminated sediments having physico-chemical properties similar to that of the test sediments. For example, to obtain a well-matched “clean” reference sample, each must have the similar grain or particle size, composition, and moisture content. Sediments can

contain a high level of natural toxicity (pore water ammonia and/or hydrogen sulphide) in areas that have high organic enrichment from natural sources (i.e. abundance of vegetable and animal life). “Clean” reference (negative control) sediment should be collected from each geographical sediment location. It is also recommended to measure pore water ammonia and hydrogen sulphide.

Sample Handling and Preservation

No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. Fill with no head space. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±3°C) using regular ice or frozen gel packs. Must not freeze during transportation.

Sample Volume

Typically one 500 mL glass jar completely filled with sediment. Sample volumes must be discussed with laboratory staff.

Stability

Store in dark at 4±2°C in airtight containers until ready for testing. It is recommended that samples are tested as soon as possible after collection. M.H.T. = 2 weeks to 6 weeks from collection.

Validity Criteria

This criteria is used to judge if the test results are valid based on the quality of embryo development in the “water-only” controls replicates and in the control sediment replicates. For a test to be considered valid, an average of ≥60% of the embryos must be normally developed larvae at the end of the test in both “water-only” controls and control sediments. This validity criteria must be met in the “water-only” controls paired and scored in conjunction with the reference toxicant as well as the sediment samples.

Quality Control

a) Reference toxicant warning charts.
b) Routine chemistry of test, reference, and control sediment samples.
c) “water-only” control and clean sediment lab control
d) Test organisms must be collected from “clean sites”.
e) Recovery success rate calculated for each treatment

Interpretation

Either:

a) The % normal larvae development for the replicate groups of test organisms exposed to the test sediment is more than 20% lower than that in the reference sediment and is significantly different ($p<0.05$).

or,

b) in the absence of a suitable reference sediment, the % normal larval development for the replicate groups of test organisms exposed to the test sediment is more than 30% lower than that in the control sediment and is significantly different ($p<0.05$).

References

a) Environment Canada, Biological Test Method: Reference Method for Measuring the Toxicity of Contaminated Sediment to Embryos and Larvae of Echinoids (Sea Urchins or Sand Dollars). Report EPS 1/RM/58 July 2014.

Revision History

March 23, 2018 BCELTAC Microbiology Toxicology Subcommittee

Polychaete Worm, *Polydora cornuta* 14d Survival and Growth Test (Marine Sediment)

Parameter	Polychaete worm growth and survival
Test Method	Biological Test Method: Test for Survival and Growth in Sediment Using Spionid Polychaete Worms (*Polydora cornuta*) Report EPS 1/RM/41 December 2001.
EMS Code	
Method Summary	The test is conducted at 23±1°C in nominally 300 mL glass beakers containing a 50 mL layer of sediment (2 cm) and 200 mL of overlying seawater. A minimum of five replicates are tested each containing five worms. The test is conducted over a duration of 14d. The survival and growth of worms is assessed at the end of the test.
Applications	Marine and estuarine sediments. Dredged materials. Pure chemicals.
Sample Considerations	Where practical and consistent with the study design and objectives, a minimum of five replicate samples (i.e. field replicates) of sediment must be taken from each discrete sampling station and depth of interest. Were practical and appropriate, sample collection must also include ≥5 samples (i.e. field replicates) from each of one or more reference stations. Reference stations should consist of sites with uncontaminated sediments having physico-chemical properties similar to that of the test sediments. For example, to obtain a well-matched “clean” reference sample, each must have the similar grain or particle size, composition, and moisture content. Sediments can contain a high level of natural toxicity (pore water ammonia and/or hydrogen sulphide) in areas that have high organic enrichment from natural sources (i.e. abundance of vegetable and animal life). “Clean” reference (negative control) sediment should be collected from each geographical sediment location. It is also recommended to measure pore water ammonia and hydrogen sulphide. Control/dilution water should have a salinity of 25–30 ppt. Suspended material and variable temperature may cause problems. Test organisms: correct species must be acclimated to dilution water. Animals must be field collected from a “clean reference” site. Note, *P. cornuta* is very sensitive to ammonia in sediments. Caution should be used to interpret results where measurable concentrations of ammonia in test sediments are present.
Sample Handling and Preservation	No preservation required. Sample volumes and frequency of material replacement must be discussed with laboratory staff. If warm (>7°C), cool to 1–7°C with regular ice or frozen gel packs upon collection; transport in the dark at 1–7°C (preferably 4±2°C) using regular ice or frozen gel packs. Must not freeze during transportation.
Sample Volume	2 L of sediment collected in a plastic pail without headspace. Sample volumes and must be discussed with laboratory staff.
Stability	Store in dark at 4±2°C until ready for testing. M.H.T. = 2 weeks to 6 weeks from collection.
Endpoints	Growth (dry weight) and survival.
Quality Control	a) Reference toxicants. b) Negative Control c) Routine chemistry of holding and dilution water.

Acceptability Criteria

The test is invalid if the following occurs**:**

The mean 14-day survival in negative control sediment <90%.

Interpretation

Results of test sediments and reference sites are usually compared using statistical methods such as ANOVA and multiple comparison tests. Reductions in the endpoints in the test sediments compared to the reference sediments may indicate the presence of toxic materials in the test sediments. A test sample may be considered to fail if the mean dry weight observed in the organisms exposed to the test sediment is >25% lower than that observed in the organisms exposed to the reference sediment and is statistically significant ($p<0.05$).

The laboratory control is used to evaluate the health and performance of the test organisms and the acceptability criteria.

References

a) Environment Canada, Biological Test Method: Test for Survival and Growth in Sediment Using Spionid Polychaete Worms (*Polydora cornuta*). Report EPS 1/RM/41 December 2001.

Revision History

March 2018 BCELTAC Microbiology Toxicology Subcommittee

Marine Sediment Bioaccumulation Test (Marine Sediment)

Parameter Marine Sediment Bioaccumulation test

EMS Code

Species	Test	Units	EMS Code
Macoma nasuta, clam	Bioaccum.	BSAF	
Nephtys caecoides, worm	Bioaccum.	BSAF	

Test Method Guidance Manual: Bedded Sediment Bioaccumulation Tests EPA/600/R-93/183, September 1993.

Method Summary The bedded sediment test is used to examine the potential for bioaccumulation of sediment contaminants into test organisms. The principle contaminants of concern are typically persistent organic compounds such as polycyclic aromatic hydrocarbons, PAH, PCBs, dioxins, and other chlorinated organics. Heavy metals such as lead, cadmium, and mercury or metalloids such as selenium may be bioaccumulative. Clean test organisms are placed into test sediments in aquaria at time 0 and are observed over the duration of the test. The duration of the test depends on the specific contaminants present in the sediments as follows:

- 28d — Metals
- 42d — PAH
- 56d — PCBs, DDT, dioxins, etc.

The test durations are based on the amount of time that is required for the contaminants to reach steady state concentrations in the exposed test organisms. For compounds where the time to reach steady state is unknown, samples of test organisms can be taken at specific time intervals and analysed to determine when steady state is reached.

Typically, the concentration of the contaminants is determined in both sediments and test organisms at time 0 and at the end of the test. The lipid content of the test organisms and the organic carbon content of the sediments should also be analysed. Additionally, the particle or grain size of sediments should be characterized.

The clam, *Macoma nasuta*, may be more suited to evaluate potential bioaccumulation of PAH because this organism metabolises PAH slowly. The worm, *Nephtys caecoides* is commonly used to evaluate the bioaccumulative potential of PCBs, dioxins and other chlorinated organics. Both test organisms may be used to evaluate metal bioaccumulation.

The test vessels or aquaria are typically 10 L or 15 L depending on sample sizes required to meet analytical detection limits.

A control is run concurrently with test organisms placed in clean sediments to evaluate control survival. Analysis of the “clean” test organisms may be useful in comparison to test sample analyses.

When whole body tissue analysis is conducted on deposit feeding organisms such as clams and worms, any contaminants associated with particles in the gut are included. Depending on the mass of sediment and the associated contaminant concentrations, the gut sediment contents can measurably increase the apparent whole-body tissue concentrations. Therefore, it is often advantageous to move a portion or all test organisms to clean water for a period of time after the test has concluded to allow the organisms to purge their guts of undigested particulate matter.

Applications Estuarine and marine sediments, dredged or waste soil material for ocean disposal.

Sample considerations

The bioaccumulation test is not designed to evaluate toxicity but to evaluate bioaccumulation potential of contaminants of concern. Therefore, this test will not be effective for sediments with high acute toxicity because test organisms may not survive over the duration of the test.

The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand, ammonia and sulphide. Field collected sediments may contain indigenous organisms including predators, and the same or closely related species. Test sediments must be sieved prior to testing to remove indigenous organisms. Control/dilution water should have a salinity of 25–30 ppt. Test organisms: correct species must be acclimated to dilution water. Test organisms must be obtained from clean reference areas.

Sample Volume

A minimum of 2.0 kg of coarse-sieved sediment is required for the test depending on test duration. For example, 56-day test requires twice as much sediment because the test sediments are renewed after 28 days. It is important to collect sufficient sample to allow for analytical samples to be taken. Sample volumes should be specified by the laboratory conducting the bioaccumulation tests.

Sample Handling and Preservation

No preservation is required. Sediments should be stored in dark at 4°±2C without headspace in sealed containers. Samples may be retested within 6 weeks of collection.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = 2 weeks to 6 weeks from collection. Freezing and longer storage might change sediment properties and should be avoided.

Endpoints

The concentrations of contaminants of concern are determined in sediments and biota to evaluate bioaccumulation potential. A biota sediment accumulation factor, BSAF is usually calculated based on the concentrations of analytes determined in the sediment and test organisms.

Acceptability Criteria

80% control survival required for valid testing. Reference and control sediments required. Statistical (ANOVA) calculation should be used to determine significant difference from control sediment.

Quality Control

a) Routine chemistry of holding and dilution water.
b) Analysis of control sediments, dilution water, and control test organisms.
c) Animals to be collected from “clean sites” and acclimated prior to testing.
d) Controls with “clean” sediment and dilution water.

Interpretation

A statistically significant difference in the tissue concentrations of a toxicant is observed between the organisms exposed to the test sediment and the organisms exposed to the reference sediment. The BSAF can indicate the potential for the contaminant(s) to bioaccumulate in the food web (trophic transfer). Specifically, the test can be used to evaluate the bioaccumulative potential of contaminants in sediment dwelling organisms. For non-regulatory purposes, comparisons to a control can be made when a suitable reference site is not available, relying on significant difference between test and control to assist in the interpretation of results (see test method for further details). Comparisons to control cannot be relied on for inclusion in the regulatory test battery since this pass/fail criterion does not provide a comparable level of environmental protection as comparisons to reference and are therefore not sufficiently protective to be used for regulatory purposes.

References

a) Guidance Manual: Bedded Sediment Bioaccumulation Tests EPA/600/R-93/183 September 1993. USEPA, September, 1993.

Revision History

March 2018 BCELTAC Microbiology Toxicology Subcommittee

Soil Toxicity Tests

Earthworm Toxicity Tests (Soil)

Parameter Earthworm toxicity tests

EMS Code

Species	Test	Units	EMS Code
E. andrei, E. fetida, L. terrestris	Acute toxicity	%, LC50	
E. andrei, E. fetida, L. terrestris	Acute avoidance	%, IC50	
E. andrei	Survival and Repro.	%, IC50	

Test Method Environment Canada Biological Test Method: Tests for Toxicity, Contaminated Soil to Earthworms (*Eisenia andrei*, *Eisenia fetida*, or *Lumbriculus terrestris* EPS 1/RM/43 — June 2004 with June 2007 Amendments.

Method Summary There are three separate tests that can be conducted with earthworms to examine the toxicity of soils to a soil invertebrate. A 14d acute toxicity test, a 48 or 72h acute avoidance test, or a 28 to 56d chronic survival and reproduction test. All test methods are performed static using one or more samples of contaminated soil. The tests can also be used to evaluate the toxicity of chemicals spiked into negative control or clean soils.

The acute lethality test is conducted for 14 days using adult or sub-adult worms. Tests are conducted in glass jars or plastic containers 500 mL to 1 L in volume. There are typically five replicates per treatment and a control. This is the most commonly used of the earthworm toxicity test methods.

The sublethal avoidance test is conducted for 48 or 72 hours with adult earthworms. It is conducted using a series of circular test units constructed of stainless steel or plexiglass. Each test unit has a circular central chamber without substrate with holes leading to each of six pie-shaped, interconnected test compartments. Three of the compartments contain the test soil and three contain clean or control soil. The number of worms in each compartment is counted following a test period of 48 or 72 hours. The worms distribute themselves throughout the units in either clean or test soil. The test uses 5 replicated test units per treatment.

The prolonged exposure test is a chronic toxicity test using *E. andrei* only. The test is run for 28d with adult worms and then the adults are counted and removed from the test vessels. The test is run for a further 28d with the progeny of the adults. Thus, effects on reproduction can be assessed.

However, it may be more effective to conduct a bioaccumulation test with earthworms rather than chronic or sublethal toxicity tests.

Applications Contaminated soils, remediated soils, composts, chemical products.

Sample considerations Earthworms will not perform well in hard subsurface soils such as clays. They are most at home in loose surficial soils with relatively high organic content.

Sample Volume The amount of soil required for the test will depend on which test option is selected. Sample volumes should be specified by the laboratory conducting the tests.

Sample Handling and Preservation No preservation is required. Soils should be collected without headspace in sealed containers. Containers must be new. Plastic bags (4mm) are often used to collect soils which are then placed into a clean plastic pails with lids and/or new clean coolers.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = ideally within two weeks of sampling and preferably within 1 week. Samples may be tested up to 6 weeks from collection, and this could be extended depending on the stability of the contaminants of concern. Freezing and longer storage might change soil properties and should be avoided.

Endpoints

Acute lethality, 14d LC50

Acute 48 or 72h avoidance, percent of live worms per treatment in each test unit (i.e. the total number of worms in the three compartments containing the same soil, for each treatment), at test end EC50 can be calculated if multiple concentrations are used.

Chronic survival and reproduction, percent survival of adults in each treatment on Day 28, number of live juveniles in each treatment on Day 56, dry weight of live juveniles in each treatment. If multiple concentrations are used, then LC50 and EC50 values for survival and reproduction can be estimated.

Acceptability Criteria

14d Acute test — invalid if mean 14d survival in negative control <90%.

Acute avoidance test **—** invalid if percent survival of worms in any test unit is <90% at test end.

Prolonged exposure test — invalid if mean 28d survival of adults in negative control soil is <90% and/or if mean reproduction rate for adults in negative control soil <3 live juveniles/adult, and/or if mean dry weight of individual live juveniles in negative control soil is <2.0 mg at test end.

Quality Control

a) Routine chemistry of soils including organic content, particle size characterization and maximum water holding capacity, MHC.
b) Analysis of control soils, and control test organisms.
c) Animals to be collected from suppliers and acclimated prior to testing.
d) Controls with “clean” negative control soils
e) Reference toxicant test is commonly performed with boric acid.

Interpretation

The test results are specific for each test depending on the number of concentrations evaluated. Typically, soils are evaluated for toxicity undiluted with multiple replicates of the sample. Therefore, percent survival, percent avoidance, or percent survival and reproduction will be the endpoints for these single concentration tests. Test sample results are compared with those of the control or reference soils. Chemical test items are usually evaluated with multiple concentrations. Multiple concentration tests will typically yield LC50 or EC50 results depending on the test option selected.

References

a) Environment Canada Biological Test Method: Tests for Toxicity, Contaminated Soil to Earthworms (*Eisenia andrei, Eisenia fetida*, or *Lumbriculus terrestris* EPS 1/RM/43 — June 2004 with June 2007 Amendments. Method Development and Applications Section, Environmental Technology Centre, Environment Canada.

Revision History

September 2018 BCELTAC Microbiology Toxicology Subcommittee

Springtail (*Collembola spp.*) Sublethal Toxicity Test (Soil)

Parameter Springtail sublethal toxicity test

EMS Code

Species	Test	Units	EMS Code
O. folsomi, F. candida,	28d Surv. & Repro.	%, LC50, IC50	
F. fimetaria, P. minuta	21d Surv. & Repro.	%, LC50, IC50	

Test Method Environment Canada Biological Test Method: Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil EPS 1/RM/47 Second edition — February 2014.

Method Summary This toxicity test is used to evaluate the toxicity of soils to a ubiquitous soil invertebrate, the springtail or Collembola. These organisms are common to soils in southern Canada and can tolerate poor soils with low organic content such as clays. The test can be used to examine effects on survival and reproduction of the test organism. The test duration is species specific, 21 days for *F. fimetaria* and *P. minuta* and 28 days for *F. candida* and *O. folsomi. F. candida* is used widely in standard laboratory toxicity tests. It has been found in British Columbia (Environment Canada, 2014). Results from tests on F. candida cannot be extrapolated to that of the other collembolan species because of differences in sensitivity.

Collembola species are relatively easy to culture in the laboratory and lab cultures are used for testing. The age of organisms for tests is species specific, *O. folsomi*, 28–31 day old; *F. candida*, 10–12 day old; *F. fimetaria*, 23–26 day old; *P. minata*, 13–14 day old.

Cultures must be age-synchronised prior to use in toxicity tests so that reproduction can be properly evaluated through enumeration of juveniles.

Single concentration tests are most commonly conducted with a minimum of three replicates of test soil and five replicates of clean, negative control soils. At the end of the test, effects on survival of adults and reproduction are assessed. Reproduction is evaluated by identifying and counting juvenile progeny in each replicate. The treatment means are then compared statistically.

Applications Contaminated soils, remediated soils, composts, chemical products.

Sample considerations Springtails will perform well on hard subsurface soils such as clays where earthworm tests will be problematic. They are most at home in loose surficial soils with relatively high organic content.

Sample Volume The amount of soil required for the test will depend on which test option is selected It is important to collect sufficient sample to allow for analytical samples to be taken. Sample volumes should be specified by the laboratory conducting the tests.

Sample Handling and Preservation No preservation is required. Soils should be collected without headspace in sealed containers. Containers must be new. Plastic bags (4mm) are often used to collect soils which are then placed into a clean plastic pails with lids and/or new clean coolers.

Stability Store in dark at 4±2°C until ready for testing. M.H.T. = ideally within two weeks of sampling and preferably within 1 week. Samples may be tested up to 6 weeks from collection, and this could be extended depending on the stability of the contaminants of concern. Freezing and longer storage might change soil properties and should be avoided.

Endpoints For contaminated soils, the total number of live adult springtails in each

replicate (i.e., in each test endpoints vessel) at test end; total number of live progeny in each replicate at test end (Day 21 for *F. fimetaria* and *P. minuta*; and Day 28 for *F. candida* and *O. folsomi*). LC50 for adult survival and EC50 for reproduction can be estimated if multiple concentrations tests are conducted. However, these are only typically used to evaluate chemical test items.

Acceptability Criteria

Invalid if mean survival of adults (first generation) in negative control soil at test end is < 70% for *F. candida* in natural soil and <80% for *F. candida* in artificial soil; < 60% for *P. minuta* in natural soil and < 70% for *P. minuta* in artificial soil; and < 70% for *O. folsomi*, and < 70% for *F. fimetaria*, regardless of soil type; invalid if mean reproduction rate for adults in negative control soil is < 100 live progeny/vessel for all four species

Quality Control

a) Routine chemistry of soils including particle size analysis, total organic carbon content (%), organic matter content (%), moisture content (%).
b) Analysis of control soils, and control test organisms.
c) Test organisms must be cultured in the laboratory and age-synchronized prior to testing.
d) Controls with “clean” negative control soils
e) Reference toxicant test is commonly performed with boric acid.

Interpretation

The test results are specific for each test depending on the number of concentrations evaluated. Typically, soils are evaluated for toxicity undiluted with multiple replicates of the sample. Therefore, percent survival, of adults and reproduction will be the endpoints for these single concentration tests. The results of the test soils are compared to those of the control or reference soils. Chemical test items are usually tested with multiple concentrations. Multiple concentration tests will typically yield LC50 or EC50 results.

References

a) Environment Canada Biological Test Method: Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil EPS 1/RM/47 Second edition — February 2014. Method Development and Applications Section, Environmental Technology Centre, Environment Canada.

Revision History

September 2018 BCELTAC Microbiology Toxicology Subcommittee

Terrestrial Plant Toxicity Test (Soil)

Parameter Terrestrial plant toxicity test

EMS Code

Species	Test	Units	EMS Code
Various species	Acute toxicity	% emerg. surv; rt. sht. length	

Test Method Environment Canada Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil EPS 1/RM/45 February 2005 (with June 2007 amendments).

Method Summary This toxicity test is used to evaluate the toxicity of soils to terrestrial plants. There are twelve species options provided including agricultural plants and wild grasses.

Agricultural species include: alfalfa, *Medicago sativa*, barley, *Hordeum vulgare*, carrot, *Daucus carota*, cucumber, *Cucumis sativus*, durum wheat, *Triticum durum*, lettuce, *Lactuca sativa,* radish, *Raphanus sativus* and tomato *Lycopersicon esculentum*.

Wild grasses are represented by: blue grama grass, *Bouteloua gracilis*, northern wheatgrass, *Elymus lanceolatus*, red clover, *Trifolium pretense*, and red fescue, *Festuca rubra*.

The test duration is 14 or 21 days (species specific) for effects on seedling emergence and plant growth (measured as shoot and root length and shoot and root dry mass). The method is conducted at 24 ± 3°C under static conditions with no renewal. Water is added to the test vessels to keep soils hydrated for the duration of the test.

Single concentration tests are most commonly conducted with a minimum of five replicates of test soil and five replicates of clean, negative control soils. Multiple concentration tests can be conducted with 3–6 replicates. Test vessels are typically 1 L polypropylene containers with 500 mL of test soil added. Full spectrum fluorescent lighting should be used with plant tests to ensure adequate growth. Humidity must be controlled in the room to ≥50%.

Applications Contaminated soils, remediated soils, composts, biosolids, sludge, chemical products.

Sample considerations The choice of test species should be done with the consideration of the land use. Agricultural species are commonly used for tests on composts, biosolids or sludges or contaminated soils obtained from agricultural regions. Wild grasses are often used in areas that are not zoned for agricultural use such as grasslands, forests, meadows, industrial areas, reclaimed mine sites, bioremediated soils, etc. Wild grasses should be selected based on species that are representative of the geographic region whenever possible. Information on species is provided in the Environment Canada test method. Ideally, a battery of tests should be conducted on both a monocot and at least one or two dicot species.

Sample Volume The amount of soil required for the test will depend on which test option is selected, single or multiple concentration. It is important to collect sufficient sample to allow for analytical samples to be taken. Sample volumes should be specified by the laboratory conducting the tests.

Sample Handling and Preservation

No preservation is required. Soils should be collected without headspace in sealed containers. Containers must be new. Plastic bags (4mm) are often used to collect soils which are then placed into a clean plastic pails with lids and/or new clean coolers.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = ideally within two weeks of sampling and preferably within 1 week. Samples may be tested up to 6 weeks from collection, and this could be extended depending on the stability of the contaminants of concern. Freezing and longer storage might change soil properties and should be avoided.

Endpoints

at test end (Day 14 or 21) Mean (± SD) percent emergence in each treatment/concentration

Mean (± SD) length of longest shoots and roots in each treatment

Mean (± SD) dry weight of shoots and roots in each treatment; if multi-concentration test EC50 for inhibition of % emergence, ICP for each of mean shoot length, root length, shoot dry weight, and root dry weight of individual plants surviving in each concentration at test end.

Acceptability Criteria

Invalid if any of the following occurs in the negative control soil at test end:

- Mean % emergence is <60% for carrot, cucumber, or tomato; <70% for alfalfa, barley, blue grama grass, lettuce, northern wheatgrass, red clover, or red fescue; <80% for durum wheat; or <90% for radish
- Mean % survival of emerged seedlings in negative control soil is <90% at test end
- Mean percentage of control seedlings exhibiting phytotoxicity or developmental abnormalities is >10%
- Mean root length is <40 mm for tomato; <70 mm for blue grama grass, red clover or red fescue; <80 mm for carrot; <100 mm for lettuce; <110 for northern wheatgrass or radish; <120 mm for alfalfa or cucumber, or <170 mm for barley; or <200 mm for durum wheat.
- Mean shoot length is <20 mm for lettuce; <30 mm for red clover; <40 mm for alfalfa; <45 mm for carrot; < 50 mm for blue grama grass, radish or tomato; <60 mm for cucumber; <80 mm for red fescue; <100 mm for northern wheatgrass; <150 mm for barley; or <160 mm for durum wheat.

Quality Control

a) Routine chemistry of soils including pH of soils, particle size analysis, total organic carbon content (%), organic matter content (%), moisture content (%). Optionally contaminants of concern, e.g., metals, polycyclic aromatic hydrocarbons, PAH, pesticides.
b) Analysis of control soils, and control test organisms.
c) Test organisms must be cultured in the laboratory and age-synchronized prior to testing.
d) Controls with “clean” negative control soils
e) Reference toxicant test is commonly performed with boric acid.

Interpretation

The test results are specific for each plant species. Effects on plants are statistically evaluated by comparing mean values of percent emergence, survival of emerged seedlings, evidence of phytotoxicity or developmental abnormalities, root and shoot length with control or reference soils. Typically, contaminated soils are evaluated for toxicity undiluted with five replicates. Chemical test items are usually tested with multiple concentrations. Multiple concentration tests will typically yield LC50 or IC50 results.

References

a) Environment Canada Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants

in Soil EPS 1/RM/45 February 2005 (with June 2007 amendments). Method Development and Applications Section, Environmental Technology Centre, Environment Canada.

Revision History September 2018 BCELTAC Microbiology Toxicology Subcommittee

Boreal Region Plant Toxicity Test (Soil)

Parameter Boreal region plant toxicity test

EMS Code

Species	Test	Units	EMS Code
Boreal species	Acute toxicity	% surv., rt. & sht. length & mass	

Test Method Environment Canada Biological Test Method: Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region EPS 1/RM/56 August 2013.

Method Summary This toxicity test is used to evaluate the toxicity of soils to terrestrial plants native to the Canadian Boreal region. There are seven species options provided including trembling aspen, *Populus tremuloides*, bluejoint reedgrass, *Calamagrostis canadiensis*, Canada goldenrod, *Solidago canadensis*, paper birch, *Betula papyrifera*, jack pine, *Pinus banksiana*, white spruce, *Picea glauca* or black spruce *Picea mariana*.

Each plant species has unique characteristics that affect its performance in toxicity tests, therefore, certain test procedures and conditions (i.e., number of seeds used to initiate a test, test duration, and test validity criteria) are modified on a species-specific basis to accommodate these requirements.

The test duration is 28, 35 or 42 days (species specific) for effects on plant growth, measured as shoot and root length and shoot and root dry mass. The tests are conducted at a mean temperature of 24 ± 3°C under static conditions with no renewal. An option is to reduce the temperature to 15 ± 3°C at night. Water is added to the test vessels to keep soils hydrated for the duration of the test.

Single concentration tests are most commonly conducted with a minimum of five replicates of test soil and five replicates of clean, negative control soils. Multiple concentration tests can be conducted with 3–6 replicates. Test vessels are typically 1 L polypropylene containers with 500 mL of test soil added. Full spectrum fluorescent lighting should be used with plant tests to ensure adequate growth. Humidity must be controlled in the room to ≥50%.

Applications Contaminated soils, remediated soils, intact soil cores, soils contaminated by brine and hydrocarbons, chemical products.

Sample considerations Choice of test species should be made with the consideration of the area where the contamination is located and the indigenous plant species. Commonly these tests species are used in northern areas or in peat bogs where boreal species are commonly found. BC's boreal region is found in the north east (15% of BC's land mass) and typical species are trembling aspen, white spruce, lodgepole pine and black spruce (B.C. Forest Facts, 2004). Information on all test species is provided in the Environment Canada test method. Time will be required for the laboratory to obtain the desired seeds and in many cases for germination and stratification of the seeds.

Sample Volume The amount of soil required for the test will depend on which test option is selected, single or multiple concentration. It is important to collect sufficient sample to allow for analytical samples to be taken. Sample volumes should be specified by the laboratory conducting the tests.

Sample Handling and Preservation

No preservation is required. Soils should be collected without headspace in sealed containers. Containers must be new. Plastic bags (4mm) are often used to collect soils which are then placed into a clean plastic pails with lids and/or new clean coolers.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = ideally within two weeks of sampling and preferably within 1 week. Samples may be tested up to 6 weeks from collection, and this could be extended depending on the stability of the contaminants of concern. Freezing and longer storage might change soil properties and should be avoided.

Endpoints

Mean (± SD) percent emergence in control soil (for test validity) at test end (Day 28, 35 or 42); mean (± SD) length of longest shoots and roots in each treatment at test end; mean (± SD) dry weight of shoots and roots in each treatment at test end; if multi-concentration test; 28, 35 or 42-day IC50 or IC25 for each of mean shoot length, root length, shoot dry weight, and root dry weight.

Acceptability Criteria

Invalid if any of the following occurs in the negative control soil at test end:

- Mean % emergence is <60% for trembling aspen, bluejoint reedgrass, Canada goldenrod, paper birch, jack pine, white spruce or black spruce.
- Mean root length is:

  <35 mm for trembling aspen;
  <17 mm for bluejoint reedgrass;
  <80 mm for Canada goldenrod;
  <53 mm for paper birch;
  <62 mm for jack pine;
  <36 mm for white spruce;
  <24 mm for black spruce

- Mean shoot length is:

  <10 mm for trembling aspen
  <35 mm for bluejoint reedgrass;
  <7 mm for Canada goldenrod;
  <26 mm for paper birch;
  <44 mm for jack pine;
  <26 mm for white spruce;
  <20 mm for black spruce.

Quality Control

a) Routine chemistry of soils including pH of soils, particle size analysis (% sand, % silt, % clay), total organic carbon content, TOC (%), organic matter content, OM (%), moisture content (%), water holding capacity, WHC, nitrogen, phosphorus, potassium, C:N ratio and CEC. Optionally, major cations and anions and contaminants of concern, e.g., metals, polycyclic aromatic hydrocarbons, PAH, pesticides.
b) Analysis of control soils, and control test organisms.
c) Test organisms must be cultured in the laboratory and age-synchronized prior to testing.
d) Controls with “clean” negative control soils
e) Reference toxicant test is commonly performed with boric acid.

Interpretation

The test results are specific for each plant species. Effects on plants are statistically evaluated by comparing mean values of percent emergence, survival of emerged seedlings, evidence of phytotoxicity or developmental abnormalities, root and shoot length with control or reference soils. Typically, contaminated soils are evaluated for toxicity undiluted with five replicates. Chemical test items are usually tested with multiple concentrations. Multiple concentration tests will typically yield LC50 or IC50 results.

References

a) Environment Canada Biological Test Method: Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region EPS 1/RM/56 August 2013. Method Development and Applications Section, Environmental Technology Centre, Environment Canada.

b) BC Forest Facts 2004, Managing the Boreal Forests in BC. BC Market Outreach Network. https://www.for.gov.bc.ca/dfn/ForestPractices/fnforest.htm

Revision History

September 2018 BCELTAC Microbiology Toxicology Subcommittee

Earthworm Bioaccumulation Test (Soil)

Parameter Earthworm Bioaccumulation test

EMS Code

Species	Test	Units	EMS Code
E. andrei, E. fetida, L. terrestris.	Bioaccumulation	BAF	

Test Method ASTM E1676 — 12 Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm *Eisenia fetida* and the Enchytraeid Potworm *Enchytraeus albidus*

Method Summary This method describes both an acute toxicity test and a bioaccumulation test that can be conducted with earthworms such as *Eisenia fetida* or the potworm, *Enchytraeus albidus*.

The acute toxicity test is conducted for 7 days for earthworms and 14 days for potworms.

The bioaccumulation test can be run for 28 days for earthworms and 42 days for potworms. The bioaccumulation test is the main test of interest in this method. The test can be run on contaminated soils, soils spiked with test items, or other soil types where bioaccumulation of contaminants is a concern. The OECD Test Guideline 317, Bioaccumulation in Terrestrial Oligochaetes is a more detailed guideline for use with spiked chemicals in soil.

Typically, the concentration of the contaminants is determined in both soils and test organisms at time 0 and at the end of the test. The lipid content of the test organisms and the organic carbon content of the soils should also be analyzed. Additionally, the particle or grain size, and water holding capacity of soils should be characterized.

The test vessels are typically 500 mL or 1 L glass jars depending on the analyte(s) and sample sizes required to meet analytical detection limits. Plastic containers could be used for inorganic compounds and some metals.

A control is run concurrently with test organisms placed in clean sediments to evaluate control survival. Analysis of the “clean” test organisms may be useful in comparison to test sample analyses.

If soils that are low in organic carbon are used, then worms may require feeding.

Earthworms should be allowed to purge their gut contents overnight on a moist filter paper in a covered petri dish. After purging, the weight of the worms must be determined in order to assess the possible decrease in biomass over the test duration and also to calculate the analyte concentrations in the worms.

Applications Contaminated soils, remediated soils, composts, chemical products.

Sample considerations Earthworms will not perform well in hard subsurface soils such as clays. They are most at home in loose surficial soils with relatively high organic content.

The bioaccumulation test is not designed to evaluate toxicity but to evaluate bioaccumulation potential of contaminants of concern. Therefore, this test will not be effective for sediments with high acute toxicity because test organisms may not survive over the duration of the test.

Sample Volume The amount of soil required for the test will depend on analytical detection limits, and the organic content of the soil. Sufficient sample should be provided to allow for analytical samples to be taken. Sample volumes should be specified by the laboratory conducting the tests.

Sample Handling and Preservation

No preservation is required. Soils should be collected without headspace in sealed containers. Containers must be new. Plastic bags (4mm) are often used to collect soils which are then placed into a clean plastic pails with lids and/or new clean coolers.

Stability

Store in dark at 4±2°C until ready for testing. M.H.T. = ideally within two weeks of sampling and preferably within 1 week. Samples may be tested up to 6 weeks from collection, and this could be extended depending on the stability of the contaminants of concern. Freezing and longer storage might change soil properties and should be avoided.

Endpoints

For the bioaccumulation test option, the concentrations of contaminants of concern are determined in soils and biota to evaluate bioaccumulation potential. A bioaccumulation factor, BAF is usually calculated based on the concentrations of analytes determined in the soil and test organisms.

Acceptability Criteria

80% control survival is required for valid testing. control soils required. Statistical (ANOVA) calculation should be used to determine significant difference from control soils.

Quality Control

a) Routine chemistry of soils including organic content, particle size characterization and maximum water holding capacity, MHC.
b) Analysis of control soils, and control test organisms.
c) Animals to be collected from suppliers and acclimated prior to testing.
d) Controls with “clean” negative control soils
e) Reference toxicant test is commonly performed with boric acid.

Interpretation

A statistically significant difference in the tissue concentrations of a toxicant is observed between the organisms exposed to the test soil and the organisms exposed to the reference sediment. The BAF can indicate the potential for the contaminant(s) to bioaccumulate in the food web (trophic transfer). Specifically, the test can be used to evaluate the bioaccumulative potential of contaminants in soil dwelling invertebrates to organisms that prey on earthworms such as birds and small mammals.

References

a) ASTM E1676-12, Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm *Eisenia fetida* and the Enchytraeid Potworm *Enchytraeus albidus*, ASTM International, West Conshohocken, PA, 2012,

b) OECD (2010), Bioaccumulation in Terrestrial Oligochaetes, Test Guideline No. 317, July 2010, OECD, Paris.

Revision History

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