

## **Section F**

# **TOXICITY TEST METHODS**

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## Fish Bioassay (Freshwater)

**Parameter** Fish bioassay

**Analytical Method** Freshwater bioassay (<10 ppt salinity)

<b>EMS Code</b>	<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>
	<i>Oncorhynchus mykiss</i>	96hrLC20*	%(v/v)	0466 X068
	(rainbow trout)	96hrLC50*	%(v/v)	<b>0461 X068</b>
		*without pH adjustment		

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

**Method Summary** Toxicity is determined by measuring the concentration of a material that is toxic to 50% of the test organisms over a 96 hour period (96HRLC50). The LT50 (lethal time to kill 50% of the organisms) is usually a single concentration of undiluted material. The LC20 is defined thus: if two or more fish die in the specified concentration (usually 100%) the sample is toxic.

**Matrix** Industrial effluents. Landfill leachates. Municipal wastewater. Agricultural runoff. Pure chemicals must have salinity less than 10 ppt.

**Interferences and Precautions** The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand, extreme temperatures, extremes in pH, extreme concentrations of suspended solids. Control/dilution water exhibiting extremes of water hardness or temperature, 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** For LC50 three 20 litre plastic cube-shaped containers or carboys. For others two 20L containers. Expel all air pockets. Ship with ice packs.

**Stability** No preservation required. Store in dark at 4°C. M.H.T. = 5 days.

**Accuracy** 10% mortality is allowed in the control. Greater than 10% mortality in the control tanks renders the bioassay 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 condition.

**Quality Control**

- Reference toxicant warning chart data on test fish.
- Routine chemistry of holding and dilution water.
- Stock history of test fish.

**References**

- a) Environment Canada, Biological Test Method: Acute Lethality Test Using Rainbow Trout. Report EPS 1/RM/9 July 1990 (May 1996 Amendments) and EPS 1/RM/13 July 1990 (Amended May 1996).

**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.

## pH Stabilization During Testing of Fish Bioassay

<b>Parameter</b>	Fish bioassay with pH Stabilization
<b>Analytical Method</b>	Freshwater bioassay with pH stabilization with CO2 Injection
<b>EMS Code</b>	To be assigned
<b>Method Summary</b>	Toxicity is determined by measuring the concentration of a material that is toxic to 50% of the test organisms over 96 hour period (96HRLC50). The LT50 is usually a single concentration of undiluted material. The pH stabilization is intended to replace the CO2 lost during aeration in order to maintain the pH throughout the testing at the same levels found in the original sample. The pH stabilization is accomplished by using the CO2 injection technique. 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 multi concentration tests.
<b>Interferences and Precautions</b>	Total ammonia must be measured in the samples 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.
<b>Sample Handling and Preservation</b>	For LC50 four 20 litre plastic collapsible containers or carboys. For others two 20L containers. Expel all air pockets. Ship with ice packs.
<b>Stability</b>	No preservation required. Store in dark at $\leq 6^{\circ}\text{C}$ . M.H.T. = 5 days.
<b>Quality Control</b>	a) Reference toxicant warning chart data on test fish. b) pH stabilized test can only be performed if the three conditions are met: Total ammonia must be measured, Failure of acute lethality reference method occurred, un-ionized ammonia concentration in the 100% test water does not equal or exceed 1.25 mg/L. c) Stock history of test fish.
<b>References</b>	a) Environment Canada, Biological Test Method: Acute Lethality Test Using Rainbow Trout. Report EPS 1/RM/9 July 1990 (May 1996 Amendments) and EPS 1/RM/13 July 1990 (Amended May 1996). b) Environment Canada, Environmental Protection Series: Procedure for pH Stabilization During the Testing of Acute Effluents to Rainbow Trout, Report EPS 1/RM50 March 2008
<b>Revision History</b>	July 2, 2008 Publication in the 2009 Laboratory Manual.

## Salmonid Early Life Stage Testing Bioassay

<b>Parameter</b>	Salmonid Early Life Stage (SEAL)
<b>Analytical Method</b>	30 day eyed egg-sac fry bioassay (a freshwater bioassay).
<b>EMS Code</b>	SEAL X391
<b>Method Summary</b>	Eyed salmonid eggs are exposed to effluents in a closed loop flow-through system, material being replaced every 96 hours. End points are percent hatched and percent survival after yolk sac absorption. ANOVA statistic is applied to determine any significant difference between control and effluents. Duration of the bioassay is 30 days or more at 10°C.
<b>Matrix</b>	Industrial effluents. Landfill and woodwaste leachates. Municipal wastewater/ storm water. Agricultural runoff. Pure chemicals.
<b>Units</b>	% (v/v)
<b>Interferences and Precautions</b>	<p>Test techniques/Equipment: Various types of incubation systems are available, e.g. Heath trays or modified Eagar eyeing-hatching containers. Testing must be conducted in the dark.</p> <p>The following sample properties may affect the test results: instability, extreme volatility, excessive oxygen demand, extreme temperature, pH or hardness, extreme concentrations of suspended solids. Salmonid eggs are only available in season and transplant approval is required. Care must be taken to ensure that eggs used for the tests are fertilized 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.</p>
<b>Sample Handling and Preservation</b>	Sample volumes and frequency of material replacement must be discussed with laboratory staff.
<b>Stability</b>	No preservation required. Store in dark at 4°C until ready for testing. M.H.T. = 5 days from collection.
<b>Accuracy</b>	None listed.
<b>Quality Control</b>	a) Reference toxicants. b) Genetic history and disease treatment of egg stock. c) Routine chemistry of holding and dilution water.
<b>References</b>	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.

**Revision History**

February 14, 1994:  
December 31, 2000:

Publication in 1994 Laboratory Manual.  
SEAM codes replaced by EMS codes. Out of  
print reference deleted. References updated.  
Units added.

## Daphnia spp. bioassay (*Daphnia magna*)

<b>Parameter</b>	Daphnia spp. bioassay								
<b>Analytical Method</b>	Freshwater bioassay (< 10 ppt salinity)								
<b>EMS Code</b>	<table><thead><tr><th><u>Species</u></th><th><u>Test</u></th><th><u>Units</u></th><th><u>EMS Code</u></th></tr></thead><tbody><tr><td><i>Daphnia magna</i></td><td>48hrLC50</td><td>%(v/v)</td><td><b>DMGC X296</b></td></tr></tbody></table>	<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>	<i>Daphnia magna</i>	48hrLC50	%(v/v)	<b>DMGC X296</b>
<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>						
<i>Daphnia magna</i>	48hrLC50	%(v/v)	<b>DMGC X296</b>						
<b>Method Summary</b>	Toxicity is determined by measuring the concentration of a material that is toxic to 50% of the test organisms over a 48 hour period.								
<b>Matrix</b>	Industrial effluents. Landfill leachates. Elutriates. Municipal wastewater. Agricultural runoff. Pure chemicals.								
<b>Interferences and Precautions</b>	The following sample properties may affect the test results: instability, extreme volatility, excessive oxygen demand, extreme temperature or 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 or temperature may cause problems.								
<b>Sample Handling</b>	500 mL new polybottle brim full. Ship cooled.								
<b>Accuracy</b>	10% mortality is allowed in control. If greater than 10% mortality is observed in control, the test is invalid. Warning charts should be prepared to test sensitivity of test organisms with recognized reference toxicants.								
<b>References</b>	<ol style="list-style-type: none"><li>Environment Canada, Biological Test Method: Acute Lethality Test Using <i>Daphnia</i> spp. Report EPS 1/RM/11, July 1990 (amended May 1996).</li><li>Environment Canada, Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to <i>Daphnia magna</i>. Report EPS 1/RM/14, July 1990 (amended May 1996).</li></ol>								
<b>Revision History</b>	<table><tr><td>February 14, 1994:</td><td>Publication in 1994 Laboratory Manual.</td></tr><tr><td>December 31, 2000:</td><td>SEAM codes replaced by EMS codes. References updated. Units added.</td></tr></table>	February 14, 1994:	Publication in 1994 Laboratory Manual.	December 31, 2000:	SEAM codes replaced by EMS codes. References updated. Units added.				
February 14, 1994:	Publication in 1994 Laboratory Manual.								
December 31, 2000:	SEAM codes replaced by EMS codes. References updated. Units added.								

## Amphipod Freshwater Sediment Bioassay (*Hyalella azteca*)

<b>Parameter</b>	Amphipod bioassay								
<b>Analytical Method</b>	Freshwater sediment bioassay, 10 day survival toxicology								
<b>EMS Code</b>	<table><thead><tr><th><u>Species</u></th><th><u>Test</u></th><th><u>Units</u></th><th><u>EMS Code</u></th></tr></thead><tbody><tr><td><i>Hyalella azteca</i></td><td>10 day survival</td><td>%(v/v)</td><td><b>HAGC X392</b></td></tr></tbody></table>	<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>	<i>Hyalella azteca</i>	10 day survival	%(v/v)	<b>HAGC X392</b>
<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>						
<i>Hyalella azteca</i>	10 day survival	%(v/v)	<b>HAGC X392</b>						
<b>Method Summary</b>	200g of sediment to 800mL of dilution water. Five replicates per set. Ten to twenty juvenile <i>H. azteca</i> per beaker. Record number dead after ten days and apply suitable statistical manipulation.								
<b>Matrix</b>	Freshwater sediment, soil and sludge. Note: Sediment ranging from >90% silt-and clay-size particles to 100% sand-size particles did not reduce survival or growth in laboratory.								
<b>Interferences and Precautions</b>	The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand. 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.								
<b>Sample Handling and Preservation</b>	1.5 kg of coarse-sieved sediment. Samples should be stored at 4°C and for no longer than two weeks before the start of testing. Freezing and longer storage might change sediment properties and should be avoided.								
<b>Stability</b>	No preservation required. Store in dark at 4°C. M.H.T. =14 days.								
<b>Accuracy</b>	80% control survival required for valid testing. Reference toxicants with warning charts required.								
<b>Quality Control</b>	a) Reference toxicant warning charts. b) Routine chemistry of holding and dilution water. c) Animals collected from "clean sites" and cultured in laboratory. d) Controls with dilution water only and with "clean control sediment".								

**References**

- a) ASTM-E 1383-92; Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates and Annexes A1. pages 1116-1138. Apr. 1992.
- b) Centre for Food Safety and Applied Nutrition and the Centre for Veterinary Medicine; Environmental Assistance Document 4.10, *Hyalella azteca* Acute Toxicity. March 1987.
- c) Environment Canada Biological Test Method: Test for Survival and Growth in Sediment Using the Freshwater Amphipod *Hyalella azteca*. EPS 1/RM/33, December 1997.

**Revision History**

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December 31, 2000:	SEAM codes replaced by EMS codes. References updated. One new reference added by Graham van Aggelen. Units added.

## Microtox™ Photobacteria Bioassay (*Photobacterium phosphoreum*)

<b>Parameter</b>	Microtox™ Bioassay												
<b>Analytical Method</b>	Fresh and marinewater photobacteria bioassay												
<b>EMS Code</b>	<table border="0" style="width: 100%;"> <thead> <tr> <th style="text-align: left;"><u>Species</u></th> <th style="text-align: left;"><u>Test</u></th> <th style="text-align: left;"><u>Units</u></th> <th style="text-align: left;"><u>EMS Code</u></th> </tr> </thead> <tbody> <tr> <td><i>Photobacterium phosphoreum</i></td> <td>*5 min. exposure</td> <td>%(v/v)</td> <td><b>0457 X393</b></td> </tr> <tr> <td><i>Photobacterium phosphoreum</i></td> <td>*15 min. exposure</td> <td>%(v/v)</td> <td><b>0458 X393</b></td> </tr> </tbody> </table> <p>*with model 500 Microtox™</p>	<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>	<i>Photobacterium phosphoreum</i>	*5 min. exposure	%(v/v)	<b>0457 X393</b>	<i>Photobacterium phosphoreum</i>	*15 min. exposure	%(v/v)	<b>0458 X393</b>
<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>										
<i>Photobacterium phosphoreum</i>	*5 min. exposure	%(v/v)	<b>0457 X393</b>										
<i>Photobacterium phosphoreum</i>	*15 min. exposure	%(v/v)	<b>0458 X393</b>										
<b>Method Summary</b>	Toxicity is determined by the concentration of material causing a 50% light decrease in the bacteria after 5 and/or 15 minutes.												
<b>Matrix</b>	Industrial effluents. Landfill leachates. Elutriates. Municipal wastewater. Agricultural run-off. Pure chemicals.												
<b>Interferences and Precautions</b>	The following sample properties may affect the test results: instability, extreme volatility, excessive oxygen demand, extreme temperature or pH, the presence of suspended solids or colour. Precautions must be taken to ensure proper handling of the test bacteria, including the correct storage temperature. Dilution water must be free of contaminants, the osmotic adjustment must not be too low and the reagents must be fresh.												
<b>Sample Handling</b>	500mL new polybottle brim full. Ship cooled.												
<b>Accuracy</b>	All manipulations of the sample and the bacteria are manual and depend on the skill of the technician in re-lyophilizing the bacteria, and in handling the micropipettor. Variability in volumes transferred by an experienced technician might contribute about 1% uncertainty in light readings. Variations in cuvettes contribute another 1% (Microbics1993).												
<b>References</b>	a) Environment Canada, Biological Test Method: Toxicity Test Using Luminescent Bacteria ( <i>Vibrio fischeri</i> ). Report EPS 1/RM/24, October 1992.												
<b>Revision History</b>	<table border="0" style="width: 100%;"> <tr> <td style="width: 50%;">February 14, 1994:</td> <td>Publication in 1994 Laboratory Manual.</td> </tr> <tr> <td>December 31, 2000:</td> <td>SEAM codes replaced by EMS codes. References updated by Graham van Aggelen. Units added. Note change in species name. Minor editing.</td> </tr> </table>	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.								
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.												

## Solid Phase Microtox™ Photobacteria Bioassay (*Photobacterium phosphoreum*)

<b>Parameter</b>	Microtox™ Bioassay								
<b>Analytical Method</b>	Solid phase photobacteria bioassay								
<b>EMS Code</b>	<table><thead><tr><th><u>Species</u></th><th><u>Test</u></th><th><u>Units</u></th><th><u>EMS Code</u></th></tr></thead><tbody><tr><td><i>Photobacterium phosphoreum</i></td><td>*5 min. exposure</td><td>%(v/v)</td><td><b>0457 X394</b></td></tr></tbody></table> <p>*EC50 (5 mm) with model 500 Microtox™</p>	<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>	<i>Photobacterium phosphoreum</i>	*5 min. exposure	%(v/v)	<b>0457 X394</b>
<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>						
<i>Photobacterium phosphoreum</i>	*5 min. exposure	%(v/v)	<b>0457 X394</b>						
<b>Method Summary</b>	Toxicity is determined by the concentration of sediment causing a 50% light decrease in the bacteria after 5 minutes of exposure.								
<b>Matrix</b>	Estuarine, fresh and marine water sediments. Terrestrial soil (i.e., landfills, contaminated soils, sludges, etc.).								
<b>Interferences and Precautions</b>	<p>Samples: To obtain well-matched replicate samples, each must have the same particle size, composition, and moisture content. Soils and sediments can contain a high level of natural toxicity in areas that support an abundance of vegetable and animal life. Reference (control) sediment should be collected from each geographical sediment series. In tests of "clean" soils and sediment, the EC50 is generally found to be at or above 2% concentration. Precautions must be taken to ensure proper handling of the test bacteria, including the correct storage temperature. Dilution water must be free of contaminants, the osmotic adjustment must not be too low and the reagents must be fresh.</p>								
<b>Sample Handling</b>	10 grams of <150 µm sediment. Ship cooled in 50 mL centrifuge tubes.								
<b>Accuracy</b>	Results must be compared to control reference sediment and site control sediment. All manipulations of the sample and the bacteria are manual and depend on the skill of the technician in rehydrating the bacteria, and in handling the micropipettor. Variability in volumes transferred by an experienced technician might contribute about 1% uncertainty in light readings. Variations in cuvettes contribute another 1% (Microbics 1993).								
<b>Quality Control</b>	Certified reference sediments (i.e., NRCC marine sediments or US EPA synthetic soil SSM).								

**References**

- a) Environment Canada, Biological Test Method: Toxicity Test Using Luminescent Bacteria (*Vibrio fischeri*). Report EPS 1/RM/24, October 1992.
- b) Bulich, A.A., Greene, M.W., Underwood, S.R.: Measurement of Soil and Sediment Toxicity to Bioluminescent Bacteria When in Direct Contact for a Fixed Time Period; Water Environment Federation 65th Annual Conference & Exposition. New Orleans, Louisiana, September 20-24 1992, pages 53-64.

**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.

## Fertilization Assay Using Echinoids (*Dendraster excentricus* and *Strongylocentrotus droebachiensis*)

<b>Parameter</b>	Echinoids fertilization assay			
<b>Analytical Method</b>	Marinewater bioassay (> 25ppt salinity), 10 min FID50.			
<b>EMS Code</b>	<u>Species</u> <i>Dendraster excentricus</i> (Sand dollar) or, <i>Strongylocentrotus droebachiensis</i> (Green sea urchin)	<u>Test</u> 10 min. FID50	<u>Units</u> %(v/v)	<u>EMS Code</u> <b>ECHI X395</b>
<b>Method Summary</b>	<b>Note:</b> 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.  Eggs and sperm are collected from the animals. Sperm is added to serial diluted concentrations of material for a timed period. Sperm is added to eggs, and chemically fixed after ten minutes. Percent unfertilized eggs is determined by microscopic examination. FID50 is the concentration causing 50% fertilization inhibition.			
<b>Matrix</b>	Industrial effluents. Landfill leachates. Municipal wastewater. Pure chemicals. Modified SWEP extracted sediments (elutriates).			
<b>Interferences and Precautions</b>	The following sample properties may affect the test results: instability, extreme volatility, excessive oxygen demand, extreme temperature or pH, extreme concentrations of suspended solids. Precautions must be taken to ensure proper handling of test organisms, including proper acclimation, correct maturity and freedom from disease. Tests must not be conducted out of season. Less than 80% fertilization in the controls is a warning of problems with the tests. Salinity adjustment with marine mix is required for effluents <25 ppt salinity.			
<b>Sample Handling and Preservation</b>	500mL polybottles brim full, shipped cooled. Unfiltered and no preservation.			
<b>Stability</b>	No preservation required. Store in dark at 4°C. M.H.T. = 5 days.			
<b>Accuracy</b>	80% control fertilization required for valid testing. Possible error in discriminating between fertilized and unfertilized eggs by inexperienced technicians.			

**Quality Control**

Reference toxicant warning charts.  
Routine chemistry of holding and dilution water.  
Animals collected from "clean sites".

**References**

- a) Environment Canada, Biological Test Method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars). Report EPS 1/RM/27, December 1992.
- b) Van Aggelen, G.C, Echinoderm Toxicity Testing: A Chronic Marine Bioassay, B.C. Ministry of Environment. 2 pages. 1988.

**Revision History**

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December 31, 2000:	SEAM codes replaced by EMS codes. Units added. Minor editing. Note added about choice of test organism based on fertile period.

## Marinewater Sediment Amphipod Bioassay (*Rhepoxynius abronius*)

<b>Parameter</b>	Amphipod marinewater sediment bioassay								
<b>Analytical Method</b>	Sediment-burrowing amphipods, 10 day burrowing and survival								
<b>EMS Code</b>	<table><thead><tr><th><u>Species</u></th><th><u>Test</u></th><th><u>Units</u></th><th><u>EMS Code</u></th></tr></thead><tbody><tr><td><i>Rhepoxynius abronius</i></td><td>10 day</td><td>%(v/v)</td><td><b>AMGC X396</b></td></tr></tbody></table>	<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>	<i>Rhepoxynius abronius</i>	10 day	%(v/v)	<b>AMGC X396</b>
<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>						
<i>Rhepoxynius abronius</i>	10 day	%(v/v)	<b>AMGC X396</b>						
<b>Method Summary</b>	175-200g of sediment is added to 750-800mL of salt water. 20 organisms added per beaker; 5 replicates per set. Burrowing and survival are recorded after 10 days.								
<b>Matrix</b>	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.								
<b>Interferences and Precautions</b>	The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand. Field collected sediments may contain indigenous organisms including predators, and the same or closely related species. 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 purchased from a collection consultant.								
<b>Sample Handling and Preservation</b>	2.0 kg of coarse-sieved sediment. Samples should be stored at 4°C and no longer than two weeks before the start of testing. Freezing and longer storage might change sediment properties and should be avoided.								
<b>Stability</b>	No preservation required. Store in dark at 4°C. M.H.T. = 14 days.								
<b>Accuracy</b>	80% control survival required for valid testing. Five replicates per set. Reference and control sediments required. Statistical (ANOVA) calculation should be used to determine significant difference from control sediment.								
<b>Quality Control</b>	Routine chemistry of holding and dilution water. Animals to be collected from "clean sites" and held in laboratory. Controls with dilution water only and with "clean control sediment".								
<b>References</b>	a) Environment Canada, Biological Test Method: Acute Test for Sediment Toxicity Using Marine or Estuarine Amphipods. Report EPS 1/RM/26, December 1992.								
<b>Revision History</b>	February 14, 1994: Publication in 1994 Laboratory Manual. December 31, 2000: SEAM codes replaced by EMS codes. Units added.								

## Marinewater Sediment Bioassay (*Macoma balthica*)

<b>Parameter</b>	<i>Macoma balthica</i> marinewater sediment bioassay			
<b>Analytical Method</b>	Sediment feeding (Tellinidae), 30 day burrowing and survival			
<b>EMS Code</b>	<u>Species</u> <i>Macoma balthica</i>	<u>Test</u> 30 day	<u>Units</u> %(v/v)	<u>EMS Code</u> <b>MBGC X397</b>
<b>Method Summary</b>	2.0 kg of sediment is added to a 35 x14 x14 cm tub. Salt water is added to cover the top of the sediment, approximately 2-3 litres. 50-100 <i>M. balthica</i> are randomly spread over the surface. Burrowing and survival are recorded after 30 days.			
<b>Matrix</b>	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.			
<b>Interferences and Precautions</b>	The following sample properties may affect the test results: extreme volatility, instability, excessive oxygen demand. Field collected sediments may contain indigenous organisms including predators, and the same or closely related species. 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 purchased from a collection consultant.			
<b>Sample Handling and Preservation</b>	2.0 kg of coarse-sieved sediment. Samples should be stored at 4°C and no longer than two weeks before the start of testing. Freezing and longer storage might change sediment properties and should be avoided.			
<b>Stability</b>	No preservation required. Store in dark at 4°C. M.H.T. = 14 days.			
<b>Accuracy</b>	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.			
<b>Quality Control</b>	Routine chemistry of holding and dilution water. Animals to be collected from "clean sites" and held in laboratory. Controls with dilution water only and with "clean control sediment".			
<b>References</b>	a) McGreer, E.R. Studies of the Bivalve, <i>Macoma balthica</i> (L) on a Mudflat Receiving Sewage Effluent and on Unpolluted Mudflat, Fraser River Estuary, British Columbia. Master Thesis, University of British Columbia, 1979.			

**Revision History**

February 14, 1994:  
December 31, 2000:

Publication in 1994 Laboratory Manual.  
SEAM codes replaced by EMS codes. Out of  
print references deleted. Units added. Minor  
editing.

## Marinewater Acute Lethality Bioassay Using Pacific Salmonids

**Parameter** Fish bioassay (Marine water)

**Analytical Method** Marinewater acute lethality bioassay using Pacific Salmonids

<b>EMS Code</b>	<u>Species</u>	<u>Test</u>	<u>Units</u>	<u>EMS Code</u>
	<i>Oncorhynchus kitsutch</i> , (Coho)	(see note)*	% Mortality	<b>0461 FA06</b>
	<i>Oncorhynchus tshawytscha</i> , (Chinook)	(see note)*	% Mortality	<b>0461 FA05</b>

\*Lethality in 100% effluent concentrations after salinity adjustment.

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

**Introduction** The intent of this procedure is to outline a bioassay method for use on those effluents and waters which have a salinity of greater than 10 parts per thousand. Salinities greater than 10 parts per thousand require the use of a suitably acclimated salmonid; for this procedure, coho (*Oncorhynchus kitsutch*) and chinook (*O. tshawytscha*) salmon are the species of choice.

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

**Test Organisms** **Species:** *Oncorhynchus kitsutch* and *O. tshawytscha* are to be used as the test species.

**Life Stage and Size:** Underyearling or juvenile life stages may be used as test fish. The average wet weight of the test fish should be between 4 to 14 grams. The length of the largest fish should not be more than twice that of the smallest in the same test. Mean ( $\pm$ SD) 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.

**Source:**

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 fish farms. Procurement and shipment of fish must be approved by the Federal-Provincial transplant committee.

Studies by Blackburn and Clarke 1987 and discussions with several DFO hatchery managers as well as studies done by the author indicate that 4 gram chinook can be acclimated to various salinities without difficulty and be suitable for testing in less than three weeks. Coho acclimate better when the mean wet weights are greater than 10 grams. If there is doubt on the health of the stocks a 24 hour sea-water challenge test should be conducted prior to salt water transfer, refer to Department of Fisheries and Oceans, Canadian Technical Report Fisheries and Aquatic Sciences No. 1515, January 1987, by Blackburn and Clarke, as an example for procedural details.

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

**Matrix****Transport and Storage:**

Transport at ambient ( $>1^\circ\text{C}$  and  $<30^\circ\text{C}$ ) temperature or at  $1^\circ$  to  $8^\circ\text{C}$  if transit time  $> 2$  days; sample should not freeze during transit; store in the dark at  $1^\circ$  to  $8^\circ\text{C}$  (preferably  $4 \pm 2^\circ\text{C}$ ); test within three days of sampling if possible; must be tested within five days of sampling.

**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 marine salt mixture or hyper brine solution\* to adjust. See above for controls. (\*freeze measured with recognized standard methods or instrumentation; i.e. Hydrometer, S-C-T meter, hand held optical refractometer.

**Receiving Water****Transport and Storage:**

Transport at ambient ( $>1^\circ\text{C}$  and  $30^\circ\text{C}$ ) temperature or at  $1^\circ$  to  $8^\circ\text{C}$  if transit time is  $>2$  days; sample should not freeze during transit; store in the dark at  $1^\circ$  to  $8^\circ\text{C}$  (preferably  $4 \pm 2^\circ\text{C}$ ); test within three days of sampling if possible; must be tested within five days of sampling.

**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.

## **Control and Dilution Water**

### **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 assessed 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 and warm the water to ensure complete dissolving of the salt mix. Note: Table salt is toxic to fish! Commercial suppliers of dry ocean salts can be obtained from any local pet store that deals with aquarium supplies. Wholesale suppliers include: Coast Pet Supplies, Richmond, ph: 270-8044; and Rolf C. Hagen, Richmond, 273-8478.

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, hyperbrine solution >40ppt, also used to adjust salinities.

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.

## **Sea Water Challenge**

### **Methods:**

Five fish are transferred directly into a glass aquarium filled with 30 litres of 25-28 ppt sea water with minimum handling. Standard aeration rates and photo period apply and testing temperature should be  $10\pm 2^{\circ}\text{C}$  (unless specified otherwise). No food is offered during the 24 - 28 hour period.

A freshwater control must be conducted concurrently.

Observe and record fish behaviour as frequently as possible during the exposure period. If no mortalities occur, transfer remaining stock and hold for two weeks before using for bioassay testing.

## **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, "Acute Lethality Test Using Threespine Stickleback", Conservation and Protection, Ottawa, Ontario, Report EPS 1/RM/10, July 1990.
- c) Environment Canada, "Acute Lethality Test Rainbow Trout", Conservation and Protection, Ottawa, Ontario, Report EPS 1/RM/9, July 1990.

**Revision History**

May 1997:

Method developed by PESC on behalf of the ministry.

December 31, 2000:

Method distributed to bioassay laboratories.  
Method inserted into main Laboratory Manual with minor reformatting and editing. EMS codes added.

<b>Table F-1 Checklist of Recommended Conditions and Procedures for Holding and Acclimating Chinook and Coho Salmon</b>	
Source of fish	cultured or obtained from fish farms; free of known diseases; procurement and transport approved by Federal/Provincial transplant committee. List of fish farms can be obtained from: Ministry of Agriculture, Fisheries and Food, Licenses Section, Aquaculture and Commercial Fisheries Branch, Courtenay, Fax: 334-1410, Ph: 334-1401.
Water	uncontaminated natural seawater or artificial seawater; holding and volume and flow, 1.0 L/10 grams of fish and 1.4L/g fish per day, respectively; ideally, salinity within 5 ppt of value for control/dilutions, for greater than 2 weeks.
Temperature	holding temperature within range compatible with good fish health acclimation temperature achieved at a rate <math><3^{\circ}\text{C}/\text{d}</math> and held at <math&gt;10\pm &gt;2="" 2^{\circ}\text{c}&lt;="" for="" math&gt;="" td="" weeks.<=""> </math&gt;10\pm>
Oxygen/aeration	dissolved oxygen 80-100% saturation; (measured with a salinity corrected instrument); maintained by aeration, filtered oil-free if necessary.
Lighting	full-spectrum fluorescent, $\leq 500$ lux at surface, ambient of fixed (16 $\pm$ 1h:8 $\pm$ 1h dark) photoperiod, preferably gradual transition between light and dark.
Feeding	at least once a day with standard commercial pelleted feed; feed stored frozen or according to manufacturer's recommendations.
Cleaning	siphoning of debris, daily or as required; transfer to clean, disinfected tanks as necessary.
Disease	mortalities monitored daily and moribund fish removed; mortality rate for group to be used $\leq 1\%$ d during seven days preceding test.
Measurement/records	temperature, dissolved oxygen, pH, salinity, flow rate and mortality should be measured and recorded preferably daily.
Sea water challenge test	prior to salt acclimation conduct test; DFO Tech report 1515 procedure as an example (with no blood chemistry).

<b>Table F- 2 Checklist for Recommended Test Conditions and Procedures (Universal)</b>	
Test type	static, 96 hour duration.
Control/dilution water	“uncontaminated” lab seawater; artificial seawater using commercial marine salt mixes; “upstream” receiving water to assess toxic effects at specific locations; dissolved oxygen content 90-100% saturation at time of use; (calibrated DO meter to reflect salinity); ideally salinity within $\pm 5$ ppt of acclimation.
Fish	underyearlings or juveniles, mean weight (Chinook 4g; coho 10g) normally a minimum 10/test solution; fish loading density $\leq 0.5$ g/L.d-1 over four days. Note: larger test volumes will be required to achieve this loading density, (i.e. > 30L final vol.).
Temperature	$10 \pm 2^\circ\text{C}$ (unless specified otherwise).
Aeration	upon preparation, pre-aerate each test solution for 30 min at $\leq 7.5$ ml/min.L-L-1; use an air flow meter (i.e. Gilmont) to calibrate air flow rate; then after, <b>and only if necessary, pre-aerate each test solution at <math>\leq 7.5</math> ml/min.L-1 for the lesser of 90 minutes of achieving <math>\geq 70\%</math> saturation in the highest test concentration;</b> aerate solutions at this rate throughout the test. Calibrate D.O. meter to compensate for salinity.
pH	no adjustment if pH of test solution within the range 6.5-8.5 (if pH is outside this; results may reflect toxicity due to biologically adverse pH); a second (pH-adjusted) test may be required or appropriate if sample/solution pH beyond this range.
Lighting	full-spectrum fluorescent, $\leq 500$ lux at surface, normally $16 \pm 1$ h light: $8 \pm 1$ dark, preferably gradual transition.
Feeding	do not feed 24 h prior to start of test, nor during test.
Observations	at least 24, 48, 72 and 96 h; for fish death, appearance and behaviour.
Measurement	solution temperature, pH and D.O.; measured at least at beginning and end (preferably daily); salinity at start.
Endpoints	as specified and/or depending on test objectives and test material; may be 96HRLC50 or single-concentration test (LT50; time to 50% mortality during 96 h.).
Reference toxicant	phenol an/or zinc (as zinc sulphate); determine static 96HRLC50 after acclimation.
Test validity	invalid if > 10% control fish die or exhibit a typical/stressed behaviour.