Section F

TOXICITY TEST METHODS

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SECTION F Toxicity Test Methods

Fish Bioassay (Freshwater)

Parameter

Fish bioassay

Analytical Method

Freshwater bioassay (<10 ppt salinity)

EMS Code

Species	Test	Units	EMS Code
Oncorhynchus mykiss	96hrLC20*	%(v/v)	0466 X068
(rainbow trout)	96hrLC50*	%(v/v)	**0461 X068**

*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

a) Reference toxicant warning chart data on test fish.
b) Routine chemistry of holding and dilution water.

c) 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.

Salmonid Early Life Stage Testing Bioassay

Parameter

Salmonid Early Life Stage (SEAL)

Analytical Method

30 day eyed egg-sac fry bioassay (a freshwater bioassay).

EMS Code

SEAL X391

Method Summary

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.

Matrix

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

Units

% (v/v)

Interferences and Precautions

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.

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.

Sample Handling and Preservation

Sample volumes and frequency of material replacement must be discussed with laboratory staff.

Stability

No preservation required. Store in dark at 4°C until ready for testing. M.H.T. = 5 days from collection

Accuracy

None listed.

Quality Control

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

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.

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
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Daphnia spp. bioassay (*Daphnia magna*)

Parameter

Daphnia spp. bioassay

Analytical Method

Freshwater bioassay (< 10 ppt salinity)

EMS Code

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

Method Summary

Toxicity is determined by measuring the concentration of a material that is toxic to 50% of the test organisms over a 48 hour period.

Matrix

Industrial effluents. Landfill leachates. Elutriates. Municipal wastewater. Agricultural runoff. Pure chemicals.

Interferences and Precautions

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.

Sample Handling

500 mL new polybottle brim full. Ship cooled.

Accuracy

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.

References

a) Environment Canada, Biological Test Method: Acute Lethality Test Using Daphnia spp. Report EPS 1/RM/11 July 1990 (amended May1996)
b) Environment Canada, Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*. Report EPS 1/RM/14 July 1990 (amended May 1996)

Revision History

February 14, 1994:	Publication in 1994 Laboratory Manual.
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Amphipod Freshwater Sediment Bioassay (*Hyalella azteca*)

Parameter

Amphipod bioassay

Analytical Method

Freshwater sediment bioassay, 10 day survival toxicology

EMS Code

Species	Test	Units	EMS Code
Hyalella azteca	10 day survival	%(v/v)	**HAGC X392**

Method Summary

200g of sediment to 800mL of dilution water. Five replicates per set. Ten to twenty juvenile *H. azteca* per beaker. Record number dead after ten days and apply suitable statistical manipulation.

Matrix

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.

Interferences and Precautions

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.

Sample Handling and Preservation

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.

Stability

No preservation required. Store in dark at 4°C. M.H.T. =14 days.

Accuracy

80% control survival required for valid testing. Reference toxicants with warning charts required.

Quality Control

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 Al. 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

February 14, 1994:	Publication in 1994 Laboratory Manual.
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*)

Parameter

Microtox™ Bioassay

Analytical Method

Fresh and marinewater photobacteria bioassay

EMS Code

Species	Test	Units	EMS Code
Photobacterium phosphoreum	*5 min. exposure	%(v/v)	**0457 X393**
Photobacterium phosphoreum	*15 min. exposure	%(v/v)	**0458 X393**

*with model 500 Microtox™

Method Summary

Toxicity is determined by the concentration of material causing a 50% light decrease in the bacteria after 5 and/or 15 minutes.

Matrix

Industrial effluents. Landfill leachates. Elutriates. Municipal wastewater. Agricultural run-off. Pure chemicals.

Interferences and Precautions

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.

Sample Handling

500mL new polybottle brim full. Ship cooled.

Accuracy

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).

References

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

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

Parameter

Microtox™ Bioassay

Analytical Method

Solid phase photobacteria bioassay

EMS Code

Species	Test	Units	EMS Code
Photobacterium phosphoreum	*5 min. exposure	%(v/v)	**0457 X394**

*EC50 (5 mm) with model 500 Microtox™

Method Summary

Toxicity is determined by the concentration of sediment causing a 50% light decrease in the bacteria after 5 minutes of exposure.

Matrix

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

Interferences and Precautions

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.

Sample Handling

10 grams of <150 µm sediment. Ship cooled in 50 mL centrifuge tubes.

Accuracy

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 relyophilizing 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).

Quality Control

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

Parameter

Echinoids fertilization assay

Analytical Method

Marinewater bioassay (> 25ppt salinity), 10 min FID50.

EMS Code

Species	Test	Units	EMS Code
Dendraster excentricus (Sand dollar) or, *Strongylocentrotus droebachiensis* (Green sea urchin)	10 min. FID50	%(v/v)	**ECHI X395**

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.

Method Summary

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.

Matrix

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

Interferences and Precautions

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.

Sample Handling and Preservation

500mL polybottles brim full, shipped cooled. Unfiltered and no preservation.

Stability

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

Accuracy

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

February 14, 1994:	Publication in 1994 Laboratory Manual.
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*)

Parameter

Amphipod marinewater sediment bioassay

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**

Method Summary

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.

Matrix

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.

Interferences and Precautions

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.

Sample Handling and Preservation

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.

Stability

No preservation required. Store in dark at 4°C. M.H.T.=14 days.

Accuracy

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.

Quality Control

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”.

References

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

Revision History	February 14, 1994:	Publication in 1994 Laboratory Manual.
	December 31, 2000:	SEAM codes replaced by EMS codes. Units added.

Marinewater Sediment Bioassay (*Macoma balthica*)

Parameter

Macoma balthica marinewater sediment bioassay

Analytical Method

Sediment feeding (Tellinidae), 30 day burrowing and survival

EMS Code

Species	Test	Units	EMS Code
Macoma balthica	30 day	%(v/v)	**MBGC X397**

Method Summary

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 M. balthica are randomly spread over the surface. Burrowing and survival are recorded after 30 days.

Matrix

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.

Interferences and Precautions

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.

Sample Handling and Preservation

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.

Stability

No preservation required. Store in dark at 4°C. M.H.T.=14 days.

Accuracy

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

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”.

References

a) McGreer, E.R. Studies of the Bivalve, *Macoma balthica* (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:	Publication in 1994 Laboratory Manual.
	December 31, 2000:	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

EMS Code

Species	Test	Units	EMS Code
Oncorhynchus kitsutch, (Coho)	(see note)*	% Mortality	**0461 FA06**
Oncorhynchu tshawystcha, (Chinook)	(see note)*	% Mortality	**0461 FA05**

*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. tshawystcha*) 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. tshawyscha* 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 (±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 Blackburne and Clarke, as an example for procedural details.

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.

Matrix

Transport and Storage:
Transport at ambient (1>1°C and <30°C) temerature or at 1° to 8°C if transit time > 2 days; sample should not freeze during transit; store in the dark at 1° to 8°C (perferably 4±2°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°C and 30°C) temperature or at 1° to 8°C if transit time is >2 days; sample should not freeze during transit; store in the dark at 1° to 8°C ((preferably 4±2°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 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 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±2°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. Method distributed to bioassay laboratories.
December 31, 2000:	Method inserted into main Laboratory Manual with minor reformatting and editing. EMS codes added.

Table F-1	**Checklist of Recommended Conditions and Procedures for Holding and Acclimating Chinook and Coho Salmon**
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 <3°C/d and held at 10±2°C for >2 weeks
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, ≤500 lux at surface, ambient of fixed (16±1h:8±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 ≤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)

Table F- 2	**Checklist for Recommended Test Conditions and Procedures (Universal)**
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 ±5 ppt of acclimation
Fish	underyearlings or juveniles, mean weight (Chinook 4g; coho 10g) normally a minimum 10/test solution; fish loading density ≤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±2°C (unless specified otherwise)
Aeration	upon preparation, pre-aerate each test solution for 30 min at ≤7.5 ml/min.L-L-1; use an air flow meter (i.e. Gilmont) to calibrate air flow rate; then after, **and only if necessary, pre-aerate each test solution at ≤7.5 ml/min.L-1 for the lesser of 90 minutes of achieving ≥70% saturation in the highest test concentration;** 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, ≤500 lux at surface, normally 16±1h light:8h±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