

TEST SPECIFIC CHECKLIST

March 1998

Test of Larval Growth and Survival Using Inland Silverside

Page 1 / 4

Parameter	Specification	Met Specifics?		
		Y	N	NA
Sample Preparation				
Filtering.....	If indigenous organisms, filter through a sieve (60 µm) (Must)
D.O. Measurement...	In each sample prior to filtering and after T° adjustment.
Pre-aeration.....	None unless D.O. is < 4 mg/L, then aerate all test solutions (Must) for a few minutes at a rate not exceeding 100 bubbles/min, until the D.O. is ≥ 4mg/L...
pH Adjustment.....	pH measured in each sample each day before new test solutions are made. . A second (pH adjusted) test might be run if pH is outside 6.0 to 9.0.....
T° Adjustment.....	T° to be measured in sample on arrival at lab. Sample adjusted to 25 ± 1°C prior to test initiation (approximately 1h).
Salinity Adjustment...	Salinity of each sample measured before starting the test. Sample adjusted to 28 - 32 g/kg using hypersaline brine (HSB) (as per EC guidance on salinity adjustment) (Must)
Test Conditions				
Test Facility.....	Isolated from general laboratory disturbances..... Instruments available to measure basic water quality variables (T°, D.O., pH, salinity) and lab prepared for other analyses.....
Test Type.....	Static renewal.....
Test Duration.....	7 days (Must)
Test T°.....	25 ± 1°C (Must)
Light Quality.....	Ambient laboratory illumination.....
Light Intensity.....	10 - 20 µE/m ² /s.....
Photoperiod.....	16 ± 1h light; 8 ± 1h dark.....
Salinity.....	28 - 32 g/kg; preferably 30 g/kg; each test solution within 1 g/kg of the control; adjust using HSB (with a salinity of 90 ± 1g/kg) or deionized water..... Nominal test conc. adjusted and reported in consideration of any salinity adjustments (Must)
D.O. Range.....	D.O. in test solutions not be permitted to fall below 4 mg/L (Must)
Aeration.....	None, unless D.O. < 4 mg/L, then aerate all chambers at a rate not exceeding 100 bubbles/min.....
Vessel Size & Type. . .	Glass chamber with sump area; borosilicate glass or non toxic disposable plastic labware..... 600 mL - 1L containers; covered during test with safety glass plates or sheet plastic (6mm thick).....
Test Volume.....	500 - 750 mL/replicate; water depth 5 cm. Minimum of 50 mL of solution per larvae (Must)
Renewal of Solution. . .	≤ 24 h for test duration (Must) 80 - 95% of solution replaced; dead brine shrimp and detritus removed; new test solution added slowly and cautiously to avoid injury to the fish.....
Dilution/Control Water.	Filtered (60 µm) uncontaminated lab seawater, reconstituted seawater, or filtered (60 µm) upstream receiving water. Salinity: 28 - 32 g/kg (Must) ; recommend 30 g/kg; salinity adjusted using aged HSB with a salinity of 90 ± 1g/kg or deionized water, distilled water or uncontaminated freshwater. Any HSB used, be from the same source as that used to adjust the salinity of the sample or test solutions (Must) Adjusted to 25 ± 1°C before use. If the test organisms have been cultured in water which is different from the test control/dilution water, a second set of controls, using culture water, is to be included in the test..... If any HSB is added to sample or test solutions to adjust salinity, the toxicity test include a set of controls prepared using only this HSB and deionized water, adjusted to the test salinity 30 ± 2 g/kg (Must) If uncontaminated receiving water used as control/dilution water, an additional lab seawater control is to be run (Must) Any test using dilution water (eg: natural seawater) which differs from this HSB control include a separate set of controls prepared using this same dilution water (Must)

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TEST SPECIFIC CHECKLIST

March 1998

Test of Larval Growth and Survival Using Inland Silverside

Page 2 / 4

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Vessel Identification.	Test chambers labeled with test conc. and replicate number.
# Test Conc.	≥ 5 plus control to calculate ICp and LC50 (Must); dilution factor ≥ 0.5.
# Replicates/Conc.	1 plus control for single conc. test.
# Organisms/Vessel.	≥ 3 per test conc. and controls (Must); 4 replicates recommended.
	Test start with equal number of replicates for each test conc. and controls.
	≥ 10 larvae randomly distributed to each test chamber on the day preceding the test (Must); 15 larvae per chamber recommended.
	The test is started by removing ~90% of the clean seawater from each test chamber and replacing with the appropriate test solution.
Vessel Randomization.	Test chambers placed in a randomized position in water bath.
	Before beginning the test, remove and replace any dead larvae from each test chamber.
Removal of Dead.	Dead organisms discarded daily during the test.
Feeding Regime.	Feed daily during test with newly hatched (< 24h old) brine shrimp nauplii (0.10 g wet weight per replicate on days 0-2; 0.15 g wet weight per replicate on days 3-6) from days 0-6; larvae not fed on day 7.
	Equal amounts of <i>Artemia</i> be fed to each replicate test chambers (Must).
Cleaning.	All non-disposable test vessels and equipment to be thoroughly cleaned and rinsed in accordance with section 5.3 (Must).
	Siphon bottom of test chamber daily immediately before test solution renewal and feeding.
Endpoints.	Mortality and growth: if multi conc. test, LC50 for mortality and ICp for mean dry weight for surviving fish (both with their 95% confidence limits) (Must).
<u>Observations & Measurements</u>				
D.O. + pH + T° + salinity.	At least at start and end (just before or immediately after renewal) of each 24-hour exposure in representative concentrations (high, medium, low, and controls) in both the fresh and used solution (Must).
Mortality.	Mortality in each test vessel determined from a count of swimming larvae at intervals of 24 h from the start until the end of test at 7 d of exposure.
	# of fish showing loss of equilibrium or abnormal swimming behaviour determined for each test vessel.
Growth.	Mean dry weight at 7 d for each test vessel.
	Fish dried immediately at 105 °C for 6 h or at 60 °C for 24 h.
	Upon removal from oven, boats moved immediately to dessicator.
	Thereafter, the boats be individually and randomly removed from the dessicator and weighed on a balance the measures consistently to 10 µg.
	Rapid weighing and standard timing among weigh boats is necessary.
<u>Test Organism</u>				
Species.	<i>Menidia beryllina</i>
Source.	From in-house cultures or commercial suppliers
	Be identified to species (Must); confirmed by a taxonomic expert.
Age.	7 to 11-days post hatch; 24h range in age.
	In a given test, all organisms be approximately the same age and be taken from the same source.
Health Criteria.	A group of organisms not be used for a test if they appear to be unhealthy, discolored, or otherwise stressed, or if mortality exceeds 10 % preceding the test; upon failure of these criteria, the entire group is to be discarded and a new group obtained (Must).
<u>Culture/Holding Conditions</u>				
T°.	25 ± 1 °C (Must); rate of change ≤ 2 °C/day for new adult fish batches.
pH.	6.0 - 9.0.
D.O.	Culture water maintained at a minimum of 4.0 mg/L; not supersaturated.

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TEST SPECIFIC CHECKLIST

Test of Larval Growth and Survival Using Inland Silverside

March 1998

Page 3 / 4

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Salinity.	28 - 32 g/kg (Must); ideally 30 g/kg; ≤ 3 g/kg change over 12 h.
Light Quality.	Ambient laboratory illumination.
Light Intensity.	10 - 20 µE/m ² /s.
Photoperiod.	16 ± 1h light; 8 ± 1h dark.
Feeding.	Adults: flake food or frozen brine shrimp twice daily and <i>Artemia</i> nauplii (< 24h old) once daily. Newly hatched fish: with about 500 rotifers <i>Brachionus plicatilis</i> per larvae per day from hatch through 4d post-hatch; on days 5 and 6, newly hatched (<12h old) <i>Artemia</i> mixed with the rotifers; after 7d, <i>Artemia</i> (< 24h old) only.
Cleaning.	Siphoning of debris daily or as required.
Culture Water.	Filtered (≤ 60 µm) uncontaminated natural seawater, or reconstituted seawater; or filtered (≤ 60 µm) receiving water. T°, D.O., pH and salinity monitored in culture tanks daily.
Morbidity/Mortality.	Adult and pre-adult fish being cultured inspected daily for signs of disease. Mortality rates and any evidence of disease recorded at least 5 d/w. Dead and moribund individuals removed immediately.
Obtaining Eggs.	If embryos, tile should be removed and placed in hatching tray.
Hatching Eggs.	Aerate tile or remove eggs from tiles and aerate in separatory funnel. Inspect incubating embryos daily. Remove and discard dead embryos or those with fungus daily.
Facility & Apparatus.	Vessels and accessories contacting organisms and culture media made of non-toxic material (Must). Culture facility located away from physical disturbances and preferably separate from test containers.
QA/QC				
Test Validity Criteria.	≥ 80% survival in controls (Must). 0.50 mg average dry weight of control larvae where test starts with 7-days old larvae and dried immediately after test termination; or 0.43 mg if fish are first preserved (not more than 7 days) in 4% formalin or 70% ethanol (Must).
Reference Toxicant.	Monthly and following the same procedure as the definitive test (Must); ideally with larvae from culture that are used in toxicity test. Standard test of 7 d with LC50 and ICp endpoints (Must). Sodium chloride, potassium chloride, cadmium chloride, copper sulfate, sodium dodecyl sulfate and potassium dichromate are suitable. Using same water as culture dilution/water.
Warning Chart.	Prepared for each reference toxicant and continually updated. Within acceptable warning limits (± 2 SD on log scale).
Sample Handling				
Sample Collection.	For off-site effluent tests, either 3 subsamples from a single sampling or ≥ 3 separate samples are collected (Must); for on-site tests, samples are collected daily and used within 24 h.
Volumes.	Volumes of 6L per day recommended.
Containers.	Non-toxic materials for sample and transport containers, new containers or thoroughly rinsed used containers.
Labeling.	Upon collection, sample containers filled, sealed and labeled/coded. Include at least sample type, source, date and time of collection and name of sample collectors.
Holding Time.	Test to be initiated within 3 d after sampling (Must); recommend within 1d.
Holding Conditions.	Keep samples cool throughout their period of transport at 4 °C using regular ice or frozen gel packs. Upon collection, if sample > 4 °C, cool to 4 °C with regular ice or frozen gel packs (not dry ice). The portion(s) of sample or subsamples required for solution renewals be stored in darkness in sealed containers without air headspace at 4 °C.

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March 1998

Test of Larval Growth and Survival Using Inland Silverside

Page 4 / 4

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Minimum Level of Reporting	Do typical test reports reflect the minimum level of reporting outlined below? (Must).....
Sample Data.....	Brief description of sample type if and as provided to the lab. Information on labeling or coding, for each sample. Date of sample/subsample collection; date and time sample(s)/subsample(s) received at test facility. For effluent or leachate, T° of sample upon receipt at lab. D.O. and pH of each sample just before its preparation and use. Dates or days during test when individual samples or subsamples used.
Test Organism.....	Species and source of organisms. Age at start of test. Any unusual appearance, behaviour, or treatment of test organisms, before their use in the test. Data showing health of organisms, including mean % mortality preceding test.
Test Facilities.....	Name and address of test laboratory. Name of person(s) performing the test. Brief description of test vessels (size, shape, type of material).
Control/Dilution Water.	Type and source of water used as control and dilution water. Type and quantity of any chemical(s) added to control or dilution water.
Test Method.....	Statement that the Environment Canada guidance document on salinity adjustment has been followed. Citation of method used and type of test. In those instances where any sample or test solutions has/have been pH adjusted, and/or is/are filtered, brief description of procedure(s)..... Description of procedure(s) for salinity adjustment of sample and dilution water..... Description of procedure for preparation of hypersaline brine..... Frequency and type of all observations and measurements made during test. Name and citation of program(s) and methods used for calculating statistical endpoints.
Test Conditions.....	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in test method document. Manner and rate of exchange of test solutions. Number, concentration, volume, and depth of solutions in test vessels, including controls. # of individuals per test vessel, and # of replicates per treatment. Brief statement (including procedure, rate, and duration) if any pre-aeration or aeration of sample or test solutions.
Test Results.....	Dates when test was started and ended. All required measurements of T°, pH, D.O. and salinity in sample and test solutions (including HSB controls and, if natural seawater has been used as dilution water, natural seawater controls), before and made during the test. . . # and % of mortality of the organisms in each test chamber, as recorded daily. Average dry weight per original fish in each test chamber..... LC50 (including the associated 95% confidence limits) for survival data and indication of quantal statistic method used; details regarding any transformation of data that was required. ICp (including the associated 95% confidence limits) for growth data and indication of quantitative statistic method used; details regarding any transformation of data that was required. Results and duration of any toxicity tests with the reference toxicant(s) performed within 30 days of the test, together with the geometric mean value (± 2 SD) for the same reference toxicant(s) as derived at the test facility in previous tests..... Anything unusual about the test, any problems encountered, any remedial measures taken.

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