

TEST SPECIFIC CHECKLIST

Test of Larval Growth and Survival Using Topsmelt

March 1998

Page 1 / 5

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 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 20 ± 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°.....	20 ± 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 should not fall below 4 mg/L.....
Aeration.....	None, unless D.O. < 4 mg/L, then aerate all chambers at a rate not exceeding 100 bubbles/min.....
Vessel Size & Type. . .	600 mL borosilicate glass beakers; covered during test with clean polyethylene plastic.....
Test Volume.....	200 mL/replicate (Must)
Renewal of Solution. . .	≤ 24 h for test duration (Must) 75% 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 ± 1 g/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 20 ± 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)
Vessel Identification. . .	Test chambers labeled with the test conc. and replicate number.....

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.

TEST SPECIFIC CHECKLIST

Test of Larval Growth and Survival Using Topsmelt

March 1998

Page 2 / 5

Parameter	Specification	Met Specifics?		
		Y	N	NA
# Test Conc.....	≥ 5 plus control to calculate ICp and LC50 (Must) ; dilution factor ≥ 0.5. 1 plus control for single conc. test.
# Replicates/Conc.....	5 replicates per test conc. and controls (Must) Test start with equal number of replicates for each test conc. and controls.
# Organisms/Vessel...	5 larvae per test chamber (Must) Larvae be randomized before placing them into the test chambers (Must)
Vessel Randomization.	Test chambers placed in a randomized position in water bath, room or incubator.....
Removal of Dead.....	Dead organisms discarded daily during the test.
Feeding Regime.....	Feed twice daily during test with newly hatched (< 24h old) brine shrimp nauplii (40 nauplii per larvae) 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>Atherinops affinis</i>
Source.....	From in-house cultures or commercial suppliers Be identified to species (Must) ; confirmed by a taxonomic expert.
Age.....	9 to 15-days post hatch. 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°.....	Adult to be held at 18 °C; rate of change ≤ 2°C/day for new adult fish batches; newly fertilized embryos held at 20 ± 1°C.
pH.....	6.0 - 9.0.
D.O.....	Culture water maintained at > 6.0 mg/L; not supersaturated.....
Salinity.....	28 - 32 g/kg (Must) ; ideally 30 g/kg; ≤ 3 g/kg change over 12 h.
Light Quality.....	Ambient laboratory illumination; 2 cool white 40 W fluorescent lamps suspended 1.25 m above the surface of each tank.

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.

TEST SPECIFIC CHECKLIST

Test of Larval Growth and Survival Using Topsmelt

March 1998

Page 3 / 5

Parameter	Specification	Met Specifics?		
		Y	N	NA
Light Intensity.	12 - 21 $\mu\text{E}/\text{m}^2/\text{s}$
Photoperiod.	14 \pm 1h light; 10 \pm 1h dark; lights on at 6:00 and off at 2:00.
Feeding.	Adults: 0.3 g of Tetramin™ flake food twice daily; supplemental feedings of krill or chopped squid are recommended. Newly hatched fish: with newly hatched (<24h old) <i>Artemia</i>
Cleaning.	Siphoning of debris daily or as required. After use, all culture materials be washed thoroughly, then rinsed with seawater before reuse.
Culture Water.	Filtered (\leq 60 μm) uncontaminated natural seawater, or reconstituted seawater; or filtered (\leq 60 μm) receiving water. Flow to culture aquaria 0.5 L/min. 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.
Acclimation.	Once in the lab, fish treated for 2 d with a general antibiotic in a separate tank, then divided among holding tanks. No more than 30 adult fish placed in each tank (100 L holding tank).
Obtaining Eggs. (from Adults)	A 1500 W immersion heater is used to provide T° spikes; T° is raised from 18 °C to 21 °C over a 12 h period, then allowed to return to 18 °C overnight; reduced current velocity is produced once daily in each tank, from 24:00 to 2:00, by turning off the circulating pump.
Hatching Eggs.	Newly fertilized embryos be placed in screen tubes set in aquaria at 20 \pm 1°C. Gentle aeration be provided (Must). Beginning about day 9, check the screen tubes daily for the presence of larvae; isolate newly-hatched larvae into a separate screen-tube at 21 °C.
Facility & Apparatus.	Remove and discard dead embryos or those with fungus daily. 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.
Larvae Transport.	1 L ziplock plastic bag; \leq 100 larvae/bag; no food to be added; oxygen aeration for 30 s prior to bag closure; refrigerate during transport using ice; keep between 15 and 18 °C.
QA/QC				
Test Validity Criteria.	\geq 80% survival in controls (Must). 0.85 mg average dry weight of control larvae where test starts with 9-days old larvae and dried immediately after test termination; or 0.72 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). Standard test of 7 d with LC50 and ICp endpoints (Must). Copper chloride recommended; 5 replicates each of 0,56, 100, 180, and 320 $\mu\text{g}/\text{L}$ total copper. LC50 for survival with copper \leq 205 $\mu\text{g}/\text{L}$ (Must). For reference toxicant test, < 25% MSD for survival and < 50% MSD for growth. If concurrent to effluent test, use embryos from the same spawn (Must); handled in the same way and test solutions delivered to the test chambers at the same time (Must); conducted at 28 to 32 g/kg using HSB adjustment.
Warning Chart.	Using same water as culture dilution/water. Prepared for each reference toxicant and continually updated. Within acceptable warning limits (\pm 2 SD on log scale). LC50 for survival within the warning limits (\pm 2 SD) of the historic reference toxicant mean (Must).

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.

TEST SPECIFIC CHECKLIST

Test of Larval Growth and Survival Using Topsmelt

March 1998

Page 4 / 5

Parameter	Specification	Met Specifics?		
		Y	N	NA
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 2L 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.
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.
Test Facilities.	Data showing health of organisms, including mean % mortality preceding test. 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.

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.

TEST SPECIFIC CHECKLIST
Test of Larval Growth and Survival Using Topsmelt

March 1998

Page 5 / 5

Parameter	Specification	Met Specifics?		
		Y	N	NA
Test Conditions (con't).	# 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.
	Dates when test was started and ended.
Test Results.	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.

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.