TEST SPECIFIC CHECKLIST

Test of Larval Growth and Survival Using Topsmelt

March 1998

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	☐	☐	☐

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# 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 *Artemia* 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)**.	☐	☐	☐
Observations & Measurements				
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.	☐	☐	☐
Test Organism				
Species.	*Atherinops affinis*.	☐	☐	☐
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)**.	☐	☐	☐
Culture/Holding Conditions				
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.	☐	☐	☐

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Light Intensity	12 - 21 µE/m^2/s	☐	☐	☐
Photoperiod	14 ± 1h light; 10 ± 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) *Artemia*	☐	☐	☐
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 (≤ 60 µm) uncontaminated natural seawater, or reconstituted seawater; or filtered (≤ 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 ± 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	☐	☐	☐
	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	☐	☐	☐
Larvae Transport	1 L ziplock plastic bag; ≤ 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	≥ 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 µg/L total copper	☐	☐	☐
	LC50 for survival with copper ≤ 205 µg/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	☐	☐	☐
	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)	☐	☐	☐
	LC50 for survival within the warning limits (± 2 SD) of the historic reference toxicant mean **(Must)**	☐	☐	☐

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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.	☐	☐	☐
	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.	☐	☐	☐

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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.	☐	☐	☐

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