TEST SPECIFIC CHECKLIST

May 1999

Toxicity Tests Using Early Life Stages of Salmonid Fish (Rainbow Trout)
(Embryo (E) test, Embryo-alevin (EA) test, Embryo-alevin-swim-up fry (EAF) test)

Parameter	Specification	Met Specifics Y	N	NA
Sample Preparation				
Filtration	Normally none; an additional (filtered sample) parallel test can be done	...	...	...
	If indigenous organisms, filter (60 µm) before use **(Must)**	...	...	...
D.O. Measurement	In each sample/subsample prior to test initiation **(Must)**	...	...	...
Pre-aeration	None unless a sample or test solution has D.O. < 60% or > 100% upon preparation, in which case pre-aerate sample or all test solutions for 30 min and if necessary for the lesser of an additional period of ≤ 90 min or until 60 - 100% saturation is achieved **(Must)**	...	...	...
	Test initiated at this point regardless of whether 60-100% is achieved **(Must)**	...	...	...
	Rate of pre-aeration minimal and controlled **(Must)**; 6.5 ± 1 mL/min·L^{-1}	...	...	...
Temp. Measurement	In each sample/subsample on arrival at lab **(Must)**	...	...	...
Temp. Adjustment	Sample/subsample adjusted to 14 ± 1°C before use **(Must)**	...	...	...
	No use of immersion heaters **(Must)**; water bath recommended	...	...	...
pH Measurement	In each sample/subsample prior to test initiation **(Must)**	...	...	...
pH Adjustment	No adjustment if pH of test solution is within range of 6.5 to 8.5	...	...	...
	A second (pH adjusted) test might be required if pH beyond this range	...	...	...
Test Conditions				
Facility	Tests isolated from general disturbance	...	...	...
	Instruments available to measure basic water quality variables (T°, D.O., pH, conductivity) and lab prepared for other analysis (ie: hardness, alkalinity, ammonia and residual chlorine) **(Must)**	...	...	...
Test Type	Static-renewal or flow-through **(Must)**	...	...	...
Test Options	Embryo (E); Embryo/alevin (EA); Embryo/alevin/swim-up fry (EAF) tests	...	...	...
Start of Test	Within 30 min immediately following a period of 5 to 20 min for dry fertilization of eggs **(Must)**	...	...	...
End of Test	E-test: ≥ 7 days after fertilization **(Must)**; EA-test: 7 days after half of the eggs in the control are seen to have hatched; EAF-test: 30 days after half of the surviving fish in the control show swim-up behaviour	...	...	...
Temperature	Daily mean of 14 ± 1°C throughout the test **(Must)**	...	...	...
Lighting	Dark until 1 week after hatching is completed, with dim or red light during solution renewals; then controlled at 100-500 lux at water surface, with 16 ± 1h light: 8 ± 1h dark, preferably with gradual transition and preferably using full-spectrum fluorescent lights	...	...	...
In-test pH	No adjustment if pH of test solution is between 6.5 to 8.5	...	...	...
Aeration	Minimal and controlled **(Must)**; ≤ 100 bubbles/min per test chamber	...	...	...
	Static-renewal: gentle aeration throughout the test	...	...	...
	Flow-through: aerate if necessary to maintain D.O. at 60-100% saturation; if aeration is used, each replicate solution is to be aerated at a similar and controlled rate **(Must)**	...	...	...
Test Apparatus	E and EA tests: 800 mL plastic beaker with solid bottom and slits in side (incubation unit) suspended in plastic pail or glass aquarium (test chamber)	...	...	...
	EAF test: plastic pail or glass aquarium	...	...	...
Solution Renewal	Static-renewal: ≥ 80% of solution replaced each day in each chamber **(Must)**	...	...	...
	Flow-through: replacement of test solutions at ≥ 0.5L/g•day **(Must)**	...	...	...
Dilution/Control Water	Uncontaminated ground, surface, dechlorinated municipal water, or reconstituted water; D.O. 90 - 100% air saturation at time of use	...	...	...
	Adjusted to 14 ± 1°C before use **(Must)**	...	...	...
	The same control/dilution water is to be used for preparing the control and all test concentrations **(Must)**	...	...	...
# Test Conc.	≥ 5 test concentrations plus a control **(Must)**	...	...	...
# Replicates/Conc.	≥ 3 replicates of each concentration including controls **(Must)**	...	...	...
	If hypothesis test (NOEC/LOEC) ≥ 4 replicates are to be used **(Must)**	...	...	...
	Equal # of replicates for each concentration including controls **(Must)**	...	...	...
# Embryos	E-test: ≥120 embryos per concentration including the control **(Must)**	...	...	...
	EA and AEF-tests: 120 to 320 embryos/concentration	...	...	...

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Parameter	Specification	Met Specifics Y	N	NA
# Embryos (con't)	Embryos are to be distributed evenly on the bottom of each unit so that they are only one layer thick and are not clumped together **(Must)**.	...	...	...
	Formal random assignment of the group of embryos in each incubation unit to particular concentrations and replicates **(Must)**.	...	...	...
Randomization	Test concentrations to be in randomized positions in the test facility **(Must)**.	...	...	...
	Throughout a test, any routine maintenance procedures are to be performed with extra care **(Must)**.	...	...	...
Handling	Before embryos reach the eyed stage, any removal of obviously dead (ie: opaque) embryos or unfertilized eggs to control fungal infection is to be done very carefully.	...	...	...
Thinning	Random removal of a number of individual test organisms from one to more replicates, to reduce crowding, maintain an acceptable loading density and/or minimize the volumes of test solution required during each renewal is not to be done at any time during an E or EA-test, nor during the embryo or alevin stages of an EAF-test **(Must)**.	...	...	...
	After thinning (EAF-test), ≥5 swim-up fry are to be present in a replicate and ≥ 2 replicates are to be available for a given concentration **(Must)**.	...	...	...
	In all tests, any obviously dead embryos, alevins or fry are to be removed as soon as they are noted, and their numbers recorded.	...	...	...
Removal of Dead	Live individuals are not removed, whether or not they are deformed **(Must)**.	...	...	...
	Each test chamber be clearly coded or labelled to identify the substance and concentration being tested, and the date and time of starting **(Must)**.	...	...	...
Vessel Labelling	E and EA-tests: No feeding.	...	...	...
Feeding	EAF-test: feed fry 4% body wt/d with commercial starter feed, ≥4 times/d, starting when half of the surviving control fish show swim-up behaviour, continuing for a 30-d exposure, but without feed in final 24 h of exposure.	...	...	...
Vessel Cleaning	All vessels, measurement devices, stirring equipment, and fish-handling equipment to be thoroughly cleaned and rinsed **(Must)**.	...	...	...
Chemical Testing	Solubilizing agent control solution be run, if used **(Must)**.	...	...	...
	Agent concentration not exceed 0.1 mL/L.	...	...	...
Endpoints	E-test: EC50 and/or EC25 for nonviable embryos.	...	...	...
	EA-test: EC50 and/or EC25 for nonviable alevins.	...	...	...
	EAF-test: EC50 and/or EC25 for nonviable individuals at swim-up; LC50 for swim-up fry; IC25 for average dry weight of surviving swim-up fry at test end.	...	...	...
Observations & Measurements				
D.O., pH, Temp.	In representative concentrations, at start and end of 24h periods in static-renewal test, or daily in flow-through tests **(Must)**.	...	...	...
Conductivity	In each new test solution before dispensing is optional.	...	...	...
Viability/Appearance	E-test: % nonviable embryos at test end **(Must)**.	...	...	...
	EA-test: % nonviable alevins, and narrative statements on delayed hatching and deformed alevins **(Must)**.	...	...	...
	EAF-test: % nonviable individuals at swim-up, mortality of fry during final 30 days, average dry weight of surviving fry at test end, and narrative statements on delayed hatching, deformed alevins, delayed swim-up, and abnormal behaviour of fry **(Must)**.	...	...	...
Test Organism				
Species	Rainbow trout *Oncorhynchus mykiss* as the source of gametes.	...	...	...
Source	Gametes obtained from a single population and source.	...	...	...
	Government hatcheries, government research stations, and private culture facilities that are known to have disease-free fish.	...	...	...
Eggs	The pool of eggs obtained from ≥ 4 females **(Must)**.	...	...	...

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Parameter	Specification	Met Specifics Y	N	NA
Eggs (con't).	Eggs that appear abnormal in any way (opaque or milky-white in colour) or which are noticeably under- or oversized in relation to the other eggs are not to be selected for the test **(Must)**.	...	...	...
	The milt obtained from ≥ 3 males **(Must)**.	...	...	...
Milt.	Pre-fertilization screening of milt for sperm mobility to be carried out to improve			
Sperm mobility.	the likelihood of good fertilization success **(Must)**.	...	...	...
	Milt with inactive sperm not to be used for fertilization **(Must)**.	...	...	...
	Fresh milt to be obtained if the milt from all sources (the 3 males) had inactive sperm **(Must)**.	...	...	...
	Dry mixing of eggs and milt for a min of 5 min and max of 20 min **(Must)**.	...	...	...
Gametes Fertilization.	Any embryos possibly damaged or injured during transfer are to be discarded **(Must)**.	...	...	...
QA/QC				
Validity Criteria.	E-test: invalid if > 30% of controls nonviable at test end **(Must)**.	...	...	...
	EA-test: invalid if > 35% of controls nonviable at test end **(Must)**.	...	...	...
	EAF-test: invalid if > 40% of controls nonviable at time of 50% swim-up of survivors **(Must)**.	...	...	...
Reference Toxicant. .	Reagent-grade phenol and/or zinc sulphate; perform as an E-test at the time that each E, EA, or EAF-test is initiated, using a portion of the same batch of fertilized eggs used to start the definitive test **(Must)**.	...	...	...
	Change solutions and monitor water quality at least every second day throughout test.	...	...	...
	EC50 determined.	...	...	...
Warning Chart.	Prepared for each reference toxicant and continually updated **(Must)**.	...	...	...
	Results acceptable if within warning limits (± 2 SD)	...	...	...
Sample Handling				
Sample Collection. . .	For off-site effluent and leachate 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.	...	...	...
Containers.	Non-toxic materials for sample and transport containers **(Must)**.	...	...	...
	New containers or thoroughly rinsed if used containers **(Must)**.	...	...	...
	Collapsible polyethylene or polypropylene containers recommended.	...	...	...
Labelling.	Upon collection, sample containers filled, sealed and labelled/coded **(Must)**. . .	...	...	...
	Include at least sample type, source, date and time of collection and name of sample collectors.	...	...	...
Holding Time.	Test to be initiated within 3 days after sampling **(Must)**.	...	...	...
	Recommend test initiation within 1 day after sampling.	...	...	...
Holding Conditions. . .	Make effort to keep samples cool throughout their period of transport **(Must)** at	...	...	...
	1 - 7°C (preferably 4 ± 2°C) using regular ice or frozen gel packs.	...	...	...
	Upon collection, if sample > 7 °C, cool to 1 - 7°C with regular ice or frozen gel packs (not dry ice) **(Must)**.	...	...	...
	Sample be kept from freezing during transport or storage **(Must)**.	...	...	..
	The portion of sample/subsamples required for solution renewals be stored in darkness in sealed containers without air head space at 4 ± 2°C **(Must)**.	...	...	...
Sample Aliquots.	Each sample or subsample in a collection container be agitated thoroughly just before pouring **(Must)**.	...	...	...
Test Report				
Sample Data.	Brief description of sample type if and as provided to the lab **(Must)**.	...	...	...
	Information on labelling or coding, for each sample/subsample **(Must)**.	...	...	...
	Date of sample/subsample collection; date and time sample(s)/subsample(s) received at test facility **(Must)**.	...	...	...
	Dates/days during test when individual samples or subsamples used **(Must)**. .	...	...	...

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Parameter	Specification	Met Specifics Y	N	NA
Sample Data (con't). .	For effluent or leachate, temperature of sample upon receipt at lab **(Must)**. . . .	...	...	...
	D.O. and pH of sample just before its preparation and use **(Must)**.	...	...	...
	Date of elutriate generation and procedure for preparation **(Must)**.	...	...	...
Test Organism.	Species and common name **(Must)**..	...	...	...
	Source of gametes or brood stock; number of female and male brood stock used for fertilization **(Must)**.	...	...	...
	Brief description of procedure for checking sperm motility **(Must)**..	...	...	...
	Description (& time interval) of procedure for fertilization of gametes **(Must)**. . .	...	...	...
	Time interval from completion of fertilization until exposure of all groups of eggs to test solutions **(Must)**..	...	...	...
	Any usual appearance/treatment of gametes/eggs, before their use **(Must)**.. . .	...	...	...
Test Facilities.	Name and address of test laboratory **(Must)**.	...	...	...
	Name of person(s) performing the test **(Must)**.	...	...	...
	Description of test chamber(s) and associated apparatus **(Must)**.	...	...	...
Control/Dilution Water.	Type(s) and source(s) of water used as control and dilution water **(Must)**.	...	...	...
	Type/quantity of any chemical(s) added to control or dilution water **(Must)**.. . . .	...	...	...
Test Method.	Citation of biological test method used **(Must)**..	...	...	...
	Mention and description of test options chosen **(Must)**.	...	...	...
	Design and description if specialized procedure used **(Must)**.	...	...	...
	Description of procedure in those instances in which a sample, subsample, or test solution has been filtered or adjusted for hardness or pH **(Must)**.	...	...	...
	Frequency/type of all observations/measurements made during test **(Must)**. . .	...	...	...
	Programs/methods used for calculating statistical endpoints **(Must)**.	...	...	...
Test Conditions.	Design and description if any deviation from or exclusion of any of the procedures and conditions specified in the test method **(Must)**.	...	...	...
	# and concentrations of test solutions including controls; volume and depth of solution in each test chamber **(Must)**..	...	...	...
	# of individuals per test chamber; # of replicates per concentration **(Must)**. . . .	...	...	...
	Presence (rate/duration) or absence of pre-aeration or aeration **(Must)**.	...	...	...
	Manner and rate of exchange of test solutions **(Must)**.	...	...	...
	Dates when test was started and ended **(Must)**.	...	...	...
	All required measurements of temperature, pH and D.O. in sample and test solutions including controls before and during the test **(Must)**.	...	...	...
Test Results.	Average # and % of nonviable embryos in each replicate and concentration (E-test), 7 days after fertilization; EC50 and 95% confidence limits **(Must)**.	...	...	...
	Average # and % of nonviable alevins in each replicate and conc., 7 days after 50% hatch in the controls (EA-test); EC50 and conf. limits; EC25 **(Must)**..	...	...	...
	Average # and % of nonviable individuals at time of 50% control swim-up, in each replicate and conc. (EAF-test); EC50 and conf. Limits; EC25 **(Must)**.. . . .	...	...	...
	# of dead fry in each conc. after 30 days of exposure with feeding, and # that started the exposure (EAF-test); LC50 and confidence limits **(Must)**.	...	...	...
	Average dry weight of fry surviving the 30-d exposure with feeding in each replicate and conc. (EAF-test); IC25 and 95% confidence limits **(Must)**.	...	...	...
	Statements on delayed hatching and deformed alevins in each concentration (EA-test); description of any apparent differences from control **(Must)**.	...	...	...
	Statements on deformed alevins, delayed swim-up, and abnormal behaviour of fry in each conc. (EAF-test); description of differences from control **(Must)**. . . .	...	...	...
	Results of E-tests with the reference toxicants, together with the geometric mean value (± 2 SD) **(Must)**.	...	...	...
	Anything unusual about the test, any deviation from these procedures, any problems encountered, any remedial measures taken **(Must)**.	...	...	...
Info Kept On-File	Do lab SOPs indicate that the information on Section 8.2 of the EPS 1/RM/28 method must be kept on file for 5 years? **(Must)**.	...	...	...
	For details, see EPS 1/RM/28, July 1998 2nd edition, section 8.2.			

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.