

# TEST SPECIFIC CHECKLIST

Revised: May 2007

## Acute Lethality Test Using Rainbow Trout (GM)

### Reference Method For Determining Acute Lethality Of Effluents To Rainbow Trout (RM)

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Note: Shaded text reflects May 2007 method amendments

Parameter	Specification	Met Specifics		
		Y	N	NA
<b>Sample Preparation</b>				
Filtering.....	Filtering of solids is not allowed <b>(Must RM)</b> .....	...	...	...
Pre-aeration.....	All test solutions and controls for 30 min at a rate of 6.5 ± 1 mL/min·L <b>through an airstone<sup>1</sup> (Must RM)</b> . Second period if D.O. in highest test concentration is < 70% or > 100% (pre-aeration continued at 6.5 ± 1mL/min·L <sup>-1</sup> until D.O. is 70 - 100% or 90 min, whichever is shorter) <b>(Must RM)</b> .....	...	...	...
Temp. Adjustment. ....	No use of immersion heaters <b>(Must RM &amp; GM)</b> ; water bath recommended....	...	...	...
pH Adjustment. ....	No pH adjustment of sample or test solutions allowed <b>(Must RM)</b> ..... No adjustment if pH of test solution is within range of 5.5 to 8.5 <b>(GM)</b> .....	...	...	...
<b>Test Conditions</b>				
Facility.....	Tests isolated from general disturbance <b>(Must RM)</b> .....	...	...	...
Test Type.....	Static <b>(Must RM)</b> .....	...	...	...
Duration.....	96h.....	...	...	...
Temperature.....	15 ± 1°C <b>(Must RM)</b> .....	...	...	...
Lighting.....	Full spectrum fluorescent; 100 - 500 lux at surface; same as that defined for acclimation <b>(Must RM)</b> .....	...	...	...
Photoperiod.....	16 ± 1h light; 8 ± 1h dark <b>(Must RM)</b> (preferably with 15-30 min transition)....	...	...	...
In-test pH.....	pH not to be adjusted during test <b>(Must RM)</b> .....	...	...	...
D.O. Range.....	70 - 100% air saturation.....	...	...	...
Aeration.....	6.5 ± 1 mL/min·L throughout test period <b>through an airstone<sup>1</sup> (Must RM)</b> .....	...	...	...
Vessel Size & Type. ....	Covered if necessary and identical for all test solutions <b>(Must RM)</b> ..... Glass, plexiglas®, polyethylene, acrylic, polypropylene or polyethylene-lined <b>(Must RM)</b> ..... Liners to be discarded after use <b>(Must RM)</b> .....	...	...	...
Test Volume.....	Depth of ≥ 15cm <b>(Must RM &amp; GM)</b> ..... Identical in all test solutions and well mixed before use <b>(Must RM &amp; GM)</b> .....	...	...	...
Renewal of Solution. ...	None <b>(Must RM)</b> .....	...	...	...
Dilution/Control Water.	Same as holding and acclimation water..... Uncontaminated ground, surface or dechlorinated municipal water..... D.O. 90-100% air saturation <b>(Must RM)</b> .....	...	...	...
# Control/Test.....	Same water used for controls and test solutions preparation <b>(Must RM &amp; GM)</b> ..... One or more control(s) for each test conducted <b>(Must RM &amp; GM)</b> ..... Use of control solution and its fish for only one toxicity test and/or one effluent sample <b>(Must RM)</b> .....	...	...	...
Vessel Labelling.....	Clearly labelled conc., date and start time <b>(Must RM)</b> .....	...	...	...
# Test Conc.....	Multi conc. test: ≥ 5 plus one or more controls <b>(Must RM)</b> ..... Highest conc. full-strength effluent, successive conc. at least 50% strength of next highest conc. <b>(Must RM)</b> ..... Single conc. test: 1 (100% test solution) plus control <b>(Must RM)</b> ..... Randomized position of test concentrations within testing facility.....	...	...	...
# Replicates/Conc.....	Only 1 vessel per conc. required, however more may be used.....	...	...	...
# Organisms/Vessel. ...	Minimum 10 fish per test concentration for single-concentration and LC50 tests <b>(Must RM)</b> .....	...	...	...
Fish handling. ....	Equal number into each solution <b>(Must RM)</b> ..... Healthy fish taken randomly from the acclimation tanks <b>(Must RM)</b> ..... Handling and transfer procedure done in such as way as to minimize stress....	...	...	...
Loading Density. ....	Random order for adding fish to each test solution..... ≤ 0.5g/L, as determined by the mean wet weight of control fish at end of test <b>(Must RM &amp; GM)</b> .....	...	...	...
Removal of Dead.....	Daily after observations <b>(Must RM)</b> .....	...	...	...
Feeding Regime.....	No feeding 16h before start of test; nor during test <b>(Must RM &amp; GM)</b> .....	...	...	...
Vessel Cleaning.....	All test vessels, measurement devices, stirring equipment and fish transfer pails thoroughly cleaned and rinsed with control/dilution water before use <b>(Must RM)</b> .....	...	...	...
Chemical Testing.....	Solvent control solution to be run, ≤ 0.5 mL/L limit <b>(GM)</b> .....	...	...	...

<sup>1</sup>Air stones acceptable for use are: (i) Aqua Fizzz, 2.5 cm length x 1.5 cm diameter, cylindrical (one use only); or (ii) AS1 silica glass, 3.8 cm length x 1.3 cm width, rectangular **(Must RM)**

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Note: Shaded text reflects May 2007 method amendments

Parameter	Specification	Met Specifics		
		Y	N	NA
Endpoint. . . . . EPS 1/RM/13 and 1/RM/9 Amendments. . . . .  <u>Observations &amp; Measurements</u> D.O., pH, Temperature. . . . . Conductivity. . . . . Appearance/Behaviour. . . . . Mortality. . . . .  Control fish Length & Weight. . . . .	Multi conc. test: Mortality (LC50-96h, 95% confidence limits) <b>(Must RM)</b> . . . . . Single conc. test: Mortality (% mortality at 96h) <b>(Must RM)</b> . . . . . Have <b>May 2007</b> amendments been incorporated into Standard Operating Procedures (SOPs)? . . . . .  At least at start and end of test in all test vessels <b>(Must RM &amp; GM)</b> . . . . . At least at start of test in all test vessels <b>(Must RM &amp; GM)</b> . . . . . Daily in all test vessels. . . . . Daily in all test vessels. . . . . All dead fish recorded and removed <b>(Must RM)</b> . . . . . Mean fork length and mean wet weight of control fish at end of test <b>(Must RM &amp; GM)</b> . . . . .	...	...	...
<u>Test Organism</u> Source. . . . .  Age. . . . . Size. . . . .  Population. . . . . Acclimation. . . . .  Test Fish disposal. . . . .	One hatchery certified "disease-free" of known diseases, with an ongoing health monitoring and certification program. . . . . Swim-up fry or fingerling. . . . . Mean weight 0.3 to 2.5 g <b>(Must RM)</b> . . . . . Length of largest fish not to be more than twice that of smallest in the same test. . . . . All fish used in a test are derived from the same population and source <b>(GM)</b> . . . . . Record of arrival date. . . . . Fish acclimated to test conditions for a period of at least 2 weeks prior to use in test at 15 ± 2°C <b>(Must RM &amp; GM)</b> . . . . . Rate of change ≤ 3°C/day <b>(GM)</b> . . . . . Acclimation period immediately preceding fish use in a test <b>(Must RM)</b> . . . . . Surviving fish used in the test to be disposed in a humane manner at end of test (e.g., overdosing with anaesthetic such as tricaine methanesulphonate) <b>(Must RM)</b> . . . . .	...	...	...
<u>Culture/Holding Conditions</u> Temperature. . . . . pH. . . . . D.O. . . . . Lighting. . . . . Photoperiod. . . . .  Water Quality. . . . .  Monitoring. . . . .	4 - 18°C. . . . . 6.0 - 8.5. . . . . 80 - 100% air saturation. . . . . Full spectrum fluorescent. . . . . 100 - 500 lux at surface. . . . . For at least 2 weeks before a test, constant 16 ± 1h light; 8 ± 1h dark <b>(Must RM)</b> . . . . . Preferably with a 15 to 30 min transition period. . . . . Uncontaminated ground, surface or dechlorinated municipal drinking water; Total Residual Chlorine ≤ 0.002 mg/L; Unionized ammonia ≤ 0.02 mg/L, nitrite ≤ 0.06 mg/L . . . . . Temperature, D.O., pH monitored daily; ammonia and nitrite monitored weekly; total residual chlorine monitored as a minimum weekly (if using dechlorinated municipal drinking water). . . . . Water flow monitored daily or weekly; individual wet weights determined at regular intervals from ≥ 10 fish removed randomly from each holding tank . . . . . Dead and moribund fish removed immediately <b>(Must GM)</b> . . . . . Mortality monitored and recorded 5 days/week minimum <b>(Must RM &amp; GM)</b> . . . . . Cumulative rate of mortality <2% during 7-day period preceding test <b>(Must RM &amp; GM)</b> . . . . .	...	...	...

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Parameter	Specification	Met Specifics		
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	<p>If cumulative mortality is 2 to 10%, acclimation be extended for at least an additional 7 days and until cumulative 7-d mortality rate of &lt;2% is achieved in the 7 day period preceding test <b>(Must RM &amp; GM)</b>.....</p> <p>Cumulative mortality &gt; 10% per week during any 7-d period makes the group of fish unacceptable for future use if deaths are caused by disease or aquatic contaminants <b>(Must RM &amp; GM)</b>.....</p>	...	...	...
Volume/Flow of water..	≥ 1.0 L/10 g of fish; ≥ 1.4 L/g fish per day <b>(Must RM)</b> .....	...	...	...
Feeding.....	At least once a day with standard commercial food pellet; 1 - 5% of wet body weight per day; as recommended by manufacturer.....	...	...	...
Cleaning.....	Siphoning of debris to eliminate buildup; tanks are to be disinfected and thoroughly rinsed with holding/acclimating water prior to introducing a new batch of fish (disinfectants such as those containing chlorinated or iodophore compounds or n-alkyldimethylbenzylammonium chloride should be used).....	...	...	...
Disease.....	If chemically treated for disease, fish not to be used for 2 weeks thereafter <b>(Must RM)</b> .....	...	...	...
<b>QA/QC</b>				
Validity Criterion.....	Test is invalid if > 10% of control fish (combined data if replicates used in test) die or exhibit atypical/stressed behaviour <b>(Must RM &amp; GM)</b> .....	...	...	...
Reference Toxicant. ...	Reagent-grade phenol and/or zinc sulphate; LC50-96h (mg/L) determined. ... Performed under the same conditions and using the same control/dilution water than the effluent test <b>(Must RM)</b> .....	...	...	...
	Performed at least once during each calendar month when an effluent is tested, and upon acclimation of a new batch of fish <b>(Must RM)</b> .....	...	...	...
	Fish used come from the same group used in effluent test <b>(Must RM)</b> .....	...	...	...
	Stock solution of phenol to be made on day of use; zinc stored in dark at pH 3-4 <b>(Must RM)</b> .....	...	...	...
	Concentrations in stock solution to be measured chemically and used to calculate LC50 if different (≥20%) from nominal concentrations.....	...	...	...
Warning Chart. ....	Prepared for each reference toxicant using LC50 results and continually updated <b>(Must RM)</b> .....	...	...	...
	LC50-96h is acceptable if within warning limits (± 2 SD on log scale).....	...	...	...
	All calculations based on log concentrations <b>(Must RM)</b> .....	...	...	...
<b>Sample Handling</b>				
Containers.....	Containers for storage/transport made of non-toxic materials <b>(Must RM &amp; GM)</b> . New or thoroughly cleaned/rinsed if used containers <b>(Must RM &amp; GM)</b> .....	...	...	...
Volume Recommended.	Single conc. test: ≥ 25 L ; Multi conc. test: ≥ 50 L.....	...	...	...
Labelling.....	Include at least sample type, source, date and time of collection and name of sampler(s) <b>(Must RM)</b> .....	...	...	...
T° measurement.....	Upon receipt of sample(s) at the laboratory, effluent t° to be measured and recorded.....	...	...	...
Holding Time.....	Test to be initiated within 5 days after sampling <b>(Must RM &amp; GM)</b> .....	...	...	...
	Recommend test initiation within 3 days after sampling.....	...	...	...
Holding Conditions....	Held in the dark at 4 ± 2°C for a brief period in full and sealed container(s) and in a refrigerated facility; or held in full sealed container(s) at 15 ± 1°C overnight if test to be started the next day <b>(Must RM)</b> .....	...	...	...
	Sample be kept from freezing <b>(Must RM)</b> .....	...	...	...
Sub-samples.....	Content of each sample container to be thoroughly agitated and combined prior to use <b>(Must RM &amp; GM)</b> .....	...	...	...
Sample Aliquots.....	Samples thoroughly agitated prior to use for preparing aliquots <b>(Must RM &amp; GM)</b> .....	...	...	...
		...	...	...
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<b>Test Report</b>	Have lab SOPs been updated to indicate amended requirement that all toxicity tests initiated (finished or not) are to be reported? <b>(Must RM)</b> . . . . .			
Sample Data. . . . .	Name and location of effluent generator <b>(Must RM)</b> . . . . . Date and time of sampling <b>(Must RM &amp; GM)</b> . . . . . Type of sample <b>(Must RM &amp; GM)</b> . . . . . Brief description of sampling point <b>(Must RM)</b> . . . . . Sampling method <b>(Must RM &amp; GM)</b> . . . . . Person collecting sample <b>(Must RM &amp; GM)</b> . . . . .			
Test Conditions. . . . .	Test type and method (e.g., single-concentration test) <b>(Must RM &amp; GM)</b> . . . . . Indication of any deviation from any must requirements <b>(Must RM &amp; GM)</b> . . . . . Name and city of testing laboratory <b>(Must RM &amp; GM)</b> . . . . . Test species <b>(Must RM &amp; GM)</b> . . . . . Person(s) performing test and verifying results <b>(Must RM)</b> . . . . . Date and time for start of definitive test <b>(Must RM &amp; GM)</b> . . . . . pH, Temperature, D.O., and conductivity of unadjusted undiluted effluent prior to test solutions preparation <b>(Must RM)</b> . . . . . Confirmation of no pH adjustment <b>(Must RM)</b> . . . . . If both pH-adjusted and non-adjusted tests are run, indication of pH adjustment procedure <b>(Must RM)</b> . . . . . Indication of pre-aeration of test solutions (rate, time) prior introduction of fish and rate of aeration throughout test <b>(Must RM &amp; GM)</b> . . . . . Concentrations and volumes tested (including controls) and indication of any replication <b>(Must RM)</b> . . . . . D.O., pH and Temperature for each test solution (including controls) at the start and end of the test; Conductivity for each test solution (including controls) at the start of the test <b>(Must RM &amp; GM)</b> . . . . .			
Fish density (length/weight). . . . .	# of fish per vessel <b>(Must RM &amp; GM)</b> . . . . . Estimated loading density (g/L); mean fork length of control fish at the end of the test, with range; mean wet weight of control fish <b>(Must RM &amp; GM)</b> . . . . .			
Results. . . . .	% mortality in fish stock tank from which test fish are taken, recorded for a minimum of 5 of the 7-d period preceding test <b>(Must RM)</b> . . . . . # of mortalities in each test solution (and controls) at 96h <b>(Must RM &amp; GM)</b> . . . . . # of control fish showing atypical/stressed behaviour <b>(Must RM &amp; GM)</b> . . . . . Mean % mortality in solutions of effluent and control water if test conducted with replicates <b>(Must RM)</b> . . . . . Mean # of control fish showing atypical/stressed behaviour if replicates used for control <b>(Must RM)</b> . . . . . Multi conc. test: LC50-96h (with 95% confidence limits, if statistically achievable) or LT50 <b>(GM)</b> and statistical method (eg: log-probit, moving average etc) on which result is based <b>(Must RM &amp; GM)</b> . . . . . Most recent LC50-96h (with 95% confidence limits) for reference toxicant(s) <b>(Must RM &amp; GM)</b> . . . . . Chemical(s) used for reference toxicant(s), date test initiated (within one month of test using the same population from which test fish were selected), historical geometric mean LC50 and warning limits ( $\pm$ 2SD) <b>(Must RM &amp; GM)</b> . . . . .			

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Parameter	Specification	Met Specifics		
		Y	N	NA
<b><u>Info Kept On-File</u></b>	Do lab SOPs indicate that the information on Section 8.2 of the EPS 1/RM/13 method must be kept on file for 5 years? <b>(Must RM)</b> . . . . .  For details of this information, see EPS 1/RM/13, section 8.2.	...	...	...

Note: In 2016, there was an amendment to the introduction section of "Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout". The amendment indicated the salinity limits of the test method, and specified that salinity is measured by refractometry. Specifications for the measurement of salinity have been described in more detail in "Reference Method for Determining Acute Lethality Using Threespine Stickleback", and "Reference Method for Determining Acute Lethality Using *Acartia tonsa*." The specifications in these more recent methods supercede the 2016 amendment to the introduction of "Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout".