

# 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> .....<br>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> .....<br>Glass, plexiglas®, polyethylene, acrylic, polypropylene or polyethylene-lined <b>(Must RM)</b> .....  | ...           | ... | ... |
| Test Volume.....            | Liners to be discarded after use <b>(Must RM)</b> .....<br>Depth of ≥ 15cm <b>(Must RM &amp; GM)</b> .....<br>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.....<br>Uncontaminated ground, surface or dechlorinated municipal water.....<br>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> .....<br>One or more control(s) for each test conducted <b>(Must RM &amp; GM)</b> .....<br>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> .....<br>Highest conc. full-strength effluent, successive conc. at least 50% strength of next highest conc. <b>(Must RM)</b> .....<br>Single conc. test: 1 (100% test solution) plus control <b>(Must RM)</b> .....<br>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> .....<br>Healthy fish taken randomly from the acclimation tanks <b>(Must RM)</b> .....<br>Handling and transfer procedure done in such as way as to minimize stress.....   | ...           | ... | ... |
| Loading Density.....        | Random order for adding fish to each test solution.....<br>≤ 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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### Reference Method For Determining Acute Lethality Of Effluents To Rainbow Trout (RM)

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

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

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## Acute Lethality Test Using Rainbow Trout (GM)

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

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| Parameter               | Specification   | Met Specifics |     |     |
|-------------------------|---|---------------|-----|-----|
|                         |   | Y             | N   | NA  |
|                         | 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 <2% is achieved in the 7 day period preceding test <b>(Must RM &amp; GM)</b> .....  | ...           | ... | ... |
|                         | Cumulative mortality > 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> .....   | ...           | ... | ... |
| 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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| Parameter                             | Specification   | Met Specifics |     |     |
|---------------------------------------|---|---------------|-----|-----|
|                                       |   | Y             | N   | NA  |
| <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> . . . . .<br>Date and time of sampling <b>(Must RM &amp; GM)</b> . . . . .<br>Type of sample <b>(Must RM &amp; GM)</b> . . . . .<br>Brief description of sampling point <b>(Must RM)</b> . . . . .<br>Sampling method <b>(Must RM &amp; GM)</b> . . . . .<br>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> . . . . .<br>Indication of any deviation from any must requirements <b>(Must RM &amp; GM)</b> . . . . .<br>Name and city of testing laboratory <b>(Must RM &amp; GM)</b> . . . . .<br>Test species <b>(Must RM &amp; GM)</b> . . . . .<br>Person(s) performing test and verifying results <b>(Must RM)</b> . . . . .<br>Date and time for start of definitive test <b>(Must RM &amp; GM)</b> . . . . .<br>pH, Temperature, D.O., and conductivity of unadjusted undiluted effluent prior to test solutions preparation <b>(Must RM)</b> . . . . .<br>Confirmation of no pH adjustment <b>(Must RM)</b> . . . . .<br>If both pH-adjusted and non-adjusted tests are run, indication of pH adjustment procedure <b>(Must RM)</b> . . . . .<br>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> . . . . .<br>Concentrations and volumes tested (including controls) and indication of any replication <b>(Must RM)</b> . . . . .<br>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> . . . . .<br>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> . . . . .<br># of mortalities in each test solution (and controls) at 96h <b>(Must RM &amp; GM)</b> . . . . .<br># of control fish showing atypical/stressed behaviour <b>(Must RM &amp; GM)</b> . . . . .<br>Mean % mortality in solutions of effluent and control water if test conducted with replicates <b>(Must RM)</b> . . . . .<br>Mean # of control fish showing atypical/stressed behaviour if replicates used for control <b>(Must RM)</b> . . . . .<br>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> . . . . .<br>Most recent LC50-96h (with 95% confidence limits) for reference toxicant(s) <b>(Must RM &amp; GM)</b> . . . . .<br>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> . . . . .<br><br>For details of this information, see EPS 1/RM/13, section 8.2. | ...           | ... | ... |