

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

Y= Yes, meets requirements; N= No, does not meet requirements; NA= not applicable

DO = dissolved oxygen; temp = temperature; conc = concentration(s); h = hour(s); min = minute(s); psi = pounds per square inch (of force); pH i = pH as measured on composite 100% sample at 15°C before any aeration of the test solutions

| TEST SPECIFIC CHECKLIST | | | | | | | |
|---|--|-----------------|---|----|----------------|---|----|
| Procedure for pH Stabilization During the Testing of Acute Lethality of Pulp and Paper Effluent to Rainbow Trout | | | | | | | |
| Parameter | Specification | Document Review | | | Implementation | | |
| | | Y | N | NA | Y | N | NA |
| General | | | | | | | |
| Purpose | pH stabilization technique (pH Controller technique) is an add-on procedure used in conjunction with instructions in EPS 1/RM/13 on samples of pulp & paper effluent (must) | | | | | | |
| | All method requirements and procedures for EPS 1/RM/13 are followed while the tests are being conducted (must) | | | | | | |
| | In the pH-stabilized sample, the pH is controlled at the level measured at test initiation (i.e., pH i) using the pH Controller technique and the single concentration test (i.e., 100% effluent) (must) | | | | | | |
| Conditions for Use | All four of the following conditions are met before the pH stabilization procedure is used (must) : | | | | | | |
| | 1) Conc of total ammonia (mg/L) is measured on pulp & paper effluent sample(s) (i.e., conc taken from 100% sample after receipt at the testing laboratory and after adjustment to 15°C) and used in the calculation of un-ionized ammonia at the initial pH (pH i) of the effluent at 15°C (must) | | | | | | |
| | 2) Un-ionized ammonia conc in 100% pulp & paper effluent is < 1.25 mg/L at 15°C, or total ammonia conc is < maximum total ammonia conc (y) in mg/L determined using the following formula at the initial pH of the pulp & paper effluent sample at 15°C: $y = 1.25 \times (10^{(9.564-pH)} + 1)$ (must) | | | | | | |
| | 3) The pH Controller technique is only used with the 100% full strength sample in conjunction with the Single Concentration test in Section 5 of EPS 1/RM/13 (must) | | | | | | |
| | 4) Any pH-stabilized test is run concurrently with another test run according to EPS 1/RM/13 (i.e., where the pH is not controlled), in parallel tests (must) ; parallel tests are initiated on the same day using the same type of exposure vessel, exposure volume, and batch of fish (must) | | | | | | |
| pH Stabilization Method | Only the pH Controller technique is used for pH stabilization during rainbow trout acute lethality testing of pulp & paper effluent samples (must) | | | | | | |

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| Total Ammonia | Measured (in mg/L) on all pulp & paper effluent samples submitted for toxicity testing using EPS 1/RM/13 (must) | | | | | | |
| Un-ionized Ammonia | Given that "total ammonia" = NH ₃ + NH ₄ ⁺ , un-ionized ammonia is calculated using the following formula (must) : un-ionized ammonia = (total ammonia) × [1/(1 + 10 ^{pK - pH})] where: - pK = 9.56 at 15°C - pH is the initial pH (pH i) of the pulp & paper effluent at 15°C - total ammonia is in mg/L as measured for Condition #1, described above | | | | | | |
| Sample Preparation | All solutions are prepared before aeration is started (must) | | | | | | |
| | Stabilization of pH starts when aeration (i.e., pre-aeration) is initiated (must) | | | | | | |
| Pre-aeration (before exposure of fish, as per EPS 1/RM/13) | Upon preparation, all test solutions and controls are pre-aerated for 30 min at a rate of 6.5 ± 1 mL/min·L (must) | | | | | | |
| | Second period of pre-aeration if DO (measured after initial 30 min. aeration) in 100% test concentration is < 70% or > 100%; pre-aeration of all solutions including control(s) is continued at 6.5 ± 1 mL/min·L until DO in the highest test concentration is 70 - 100% or 90 additional min., whichever is shorter (must) | | | | | | |
| | Fish are randomly placed in test solutions and test initiated immediately after pre-aeration regardless of whether 70 - 100% aeration achieved (must) | | | | | | |
| Air Delivery | Clean air stones are used for delivery of compressed air (must) | | | | | | |
| | Glass pipettes are used for delivery of CO ₂ gas in pH Controller technique (highly recommended) | | | | | | |
| Initial Observations & Measurements | | | | | | | |
| Initial pH, Total Ammonia, Alkalinity | pH and total ammonia are measured in each full strength effluent sample after sub-samples (aliquots of a sample divided between two or more containers) have been combined, thoroughly mixed, and adjusted to 15 ± 1°C (must) ; Alkalinity is also measured | | | | | | |
| | Detection limit for total ammonia is 0.05 mg/L | | | | | | |
| | Precision and accuracy for the total ammonia measurement is ± 20% | | | | | | |
| | Un-ionized ammonia conc is calculated using the formula provided in Section 1.2, the total ammonia measurement at 15°C and initial pH (pH i) of the sample before any aeration of test solutions (must) | | | | | | |
| | pH stabilization technique is not used if this conc of un-ionized ammonia is ≥ 1.25 mg/L (must) | | | | | | |

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| pH Controller Technique | | | | | | | |
| pH Control | Upward drift of pH is controlled by aerating pulp & paper test solutions (including control) using pure CO ₂ (or a mixture of 15% CO ₂ , 21% O ₂ , and 64% N ₂) with separate lines for laboratory air addition; CO ₂ addition is regulated by a controller that is triggered by a drift in pH above a programmed set point and shut off when pH returns to the acceptable limit (must) | | | | | | |
| Setup for pH Controller Technique | Apparatus for Controller Technique is set up as described in Section 2.2.1 and Figures 1 to 6 of EPS 1/RM/59; i.e., CO ₂ is delivered to test vessels from a compressed gas cylinder containing CO ₂ , via gas cylinder regulators, and individual pressure regulators with needle valve assemblies, connected to the gauge assembly (manifold); solenoids, are used to control the flow of CO ₂ ; and pH controllers are used to monitor and regulate CO ₂ delivery through backflow valves and glass pipettes | | | | | | |
| | Oil or grease is not used on any regulator or cylinder fittings (must) | | | | | | |
| | All solenoids are turned off before the valve on the CO ₂ cylinder is opened | | | | | | |
| | Valve on CO ₂ cylinder is opened and pressure adjusted to ~40 psi | | | | | | |
| | Working pressure on solenoid is adjusted to 20 psi (i.e., solenoid regulator gauge reads 20 psi) | | | | | | |
| | Connections are tested for leaks using liquid leak detector, and the system rechecked and sealed as required | | | | | | |
| pH Controller | pH Controller is calibrated at the start of the test and verified daily using certified pH standards (must) ; pH readings in pH-stabilized test are verified using the meter/probe used for the unstabilized test | | | | | | |
| | Sensitivity of pH Controller (i.e., tolerance) is set before test initiation (± 0.2 pH units) (must) ; typically ± 0.1 pH units | | | | | | |
| | CO ₂ tubing is removed from the exposure solution during calibration (must) | | | | | | |
| | Meter calibration is completed rapidly to prevent pH drift (must) | | | | | | |
| | Instructions for calibration and maintenance provided by manufacturer are reviewed before test initiation | | | | | | |
| | One pH probe and controller is used for each test solution for test duration | | | | | | |
| | Probe is secured 3 - 5 cm below the surface of the test solution | | | | | | |
| | CO ₂ delivery pipette is directly beneath the pH probe (for accurate pH control) | | | | | | |
| | Back-flow is prevented using spring-loaded (stainless steel) back-flow check valves | | | | | | |
| | Durable pH probes are used to reduce risk of electrode-filling solution (e.g., KCl) leaks | | | | | | |
| | Test is discontinued in the event of a probe leak (must) | | | | | | |

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| pH Controller cont. | Frequent pH measurements and adjustment of CO ₂ flow (i.e., to ensure stabilization of pH) are carried out during first few hours of the test | | | | | | |
| | pH values on the controllers are closely monitored to ensure proper operation of the solenoids (must) ; controller cycles on and off to control the flow of CO ₂ ; if solenoid remains open, CO ₂ flow is gradually increased until required pH value is reached and the solenoid closes | | | | | | |
| Flow Rates | Aeration rate for delivery of laboratory air (using oil-free compressed air) through air stone is 6.5 ± 1 mL/min · L throughout test in all exposures and control (must) ; note that addition of CO ₂ will slightly increase aeration rate when pH Controller cycles on | | | | | | |
| | Airflow meters are verified by the laboratory according to industry accepted techniques and practices for air delivery rate (i.e., positive-displacement) (must) | | | | | | |
| | Airflow meters are visually inspected prior to use and daily while in use (must) | | | | | | |
| | If aeration rates are suspected to be outside of the required range in any test vessel, the aeration rates are immediately verified and adjusted as needed (must) | | | | | | |
| | Visual checks are made once daily to ensure pH Controllers and air lines are working properly (must) | | | | | | |
| pH Measurements | pH is measured and recorded immediately before any aeration (pH _i), at t = 0 h (test start, when fish are introduced), and at t = 24, 48, 72, and 96 h in 100% sample and control (must) ; more frequent pH measurements (i.e., during first 8 hours, or twice daily) are carried out as needed | | | | | | |
| | pH measurements are carried out at the same frequency in both the unstabilized (EPS 1/RM/13) and pH-stabilized tests (100% concentration and controls) to allow for a full comparison of results (must) | | | | | | |
| | pH is also measured and recorded any time the CO ₂ flow is manually adjusted or there is a change to the set point on a pH controller, and a subsequent pH reading is taken within 30 minutes after the adjustment (must) | | | | | | |
| Ammonia Measurements | Total ammonia (in the 100% sample) is measured at test completion (96 h) and/or anytime during the test when > 50% mortality is observed (must) | | | | | | |
| | Total ammonia measurements are conducted simultaneously in both the unstabilized (EPS 1/RM/13) and pH-stabilized tests (100% concentration) (must) | | | | | | |
| | Consistent sample collection, storage, preservation, and analytical techniques are used for samples collected and analysed for total ammonia (must) | | | | | | |

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| Ammonia Measurements cont. | Storage and preservation conditions are carefully considered when collecting samples for ammonia analysis (i.e., methods are appropriate and reliable for use with pulp & paper effluents and consider possible interferences) (must) | | | | | | |
| | Analytical method selected for ammonia measurements takes into consideration the predicted/expected concentration of total ammonia in each sample and the existence of potential interferences (i.e., method is appropriate for use with pulp & paper effluent samples) | | | | | | |
| Validity Criteria | A test is considered invalid if any of the following occur (must) : | | | | | | |
| | 1) The average pH in pH stabilized 100% pulp & paper effluent test solution shifts more than ± 0.2 units from pH i (must) | | | | | | |
| | 2) The instantaneous pH in the pH stabilized 100% pulp & paper effluent test solution is greater than ± 0.3 units from pH i (must) | | | | | | |
| | 3) If >10% of the fish (combined data if replicates are used) in the pH stabilized control die or exhibit atypical or stressed behaviour (must) | | | | | | |
| Test Report | | | | | | | |
| General | In addition to reporting requirements outlined in EPS 1/RM/13, the following information is reported when conducting a pH stabilized test with pulp & paper effluent (must) | | | | | | |
| Reporting Requirements | Percentage of CO ₂ gas mix or CO ₂ used during test (must) | | | | | | |
| | pH i, total ammonia, and (if measured) alkalinity; all measured in the 100% effluent sample, after all effluent to be used in testing has been composited, thoroughly mixed, and temp of the sample adjusted to $15 \pm 1^\circ\text{C}$ (must) | | | | | | |
| | Confirmation that airflow meters were verified; visually inspected prior to use and daily when in use; if aeration rates suspected to be outside range, confirmation that aeration rates were verified and adjusted (must) | | | | | | |
| | Calculated un-ionized ammonia conc, based on the measurement of total ammonia, a temp of 15°C and the pH i of the 100% effluent sample (must) | | | | | | |
| | pH measurements taken at a minimum of $t = 0$ (test start, when fish are introduced) and at 24, 48, 72, and 96 h in the control and 100% conc (must) | | | | | | |
| | Any additional pH measurements and the time taken (must) | | | | | | |
| | Total ammonia (in the 100% sample) measured at test completion (96 h), and/or at any time during the test when > 50% mortality is observed (must) | | | | | | |
| | Calculated un-ionized ammonia concentrations corresponding to all measurements of both pH and total ammonia (must) | | | | | | |
| Coinciding pH and total ammonia measurements in the parallel EPS 1/RM/13 test (must) | | | | | | | |

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| Reporting Requirements cont. | For total ammonia measurements, description of sample collection, storage and preservation techniques, analysis method, and detection limit (with precision and accuracy to be held on file) (must) | | | | | | |
| | Average pH based on all readings in the 100% effluent measured during testing (must) | | | | | | |

Notes: