

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: