

TEST SPECIFIC CHECKLIST

Prepared: March 2009

pH Stabilization Procedure for Testing Acute Lethality of Effluent to Rainbow Trout

Page 1 / 5

Parameter	Specification	Met Specifics?		
		Y	N	NA
General	pH stabilization techniques are add-on procedures used in conjunction with EPS 1/RM/13 on samples of wastewater effluent (Must)
Conditions for Use . . .	All three of the following conditions are met before any pH stabilization procedures are used (Must) :
	1) Concentration of total ammonia is measured on wastewater effluent sample and used in the calculation of un-ionized ammonia at the initial pH (pHi) of the effluent at 15°C (Must)
	2) Wastewater effluent sample previously collected from the same source failed (i.e., > 50% mortality) the rainbow trout acute lethality test (EPS 1/RM/13) (Must)
	3) Un-ionized ammonia concentration in 100% wastewater is <1.25 mg/L at 15°C, or total ammonia concentration is < maximum total ammonia concentration (y) in mg/L determined using the following formula at the initial pH of the wastewater effluent sample at 15°C: $y = 1.25 (10^{(9.564136638 - \text{pH})} + 1) \text{(Must)}$
pH stabilization methods	One of three techniques for pH stabilization is used to control the pH of the sample at the level measured at test initiation (pH i): (1) CO ₂ Injection, (2) Recycling, and (3) pH Controller (Must)
Total Ammonia	Measured (in mg/L) on all wastewater 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) x [1/(1 + 10 ^{pK - pH})] where: - pK = 9.56 at 15°C - pH is the initial pH (pHi) of the wastewater effluent at 15°C - total ammonia is in mg/L as measured for Condition #1, described above
Sample Preparation	All solutions prepared before aeration started (Must) Stabilization of pH starts when pre-aeration initiated (Must)
Pre-aeration (as per EPS 1/RM/13)	Upon preparation, all test solutions and controls for 30 min at a rate of 6.5 ± 1 mL/min-L (Must) Second period if D.O.(measured after initial 30 min. pre-aeration) in highest test concentration (normally 100% effluent) is < 70% or >100%; pre-aeration of all solutions including controls is continued at 6.5 ± 1 mL/min-L until D.O. is 70 - 100% or 90 min., whichever is shorter (Must) Fish randomly placed in test solutions and test initiated after pre-aeration regardless of whether 70 - 100% aeration achieved (Must)
Air Delivery	Clean airstones used for delivery of CO ₂ mix for CO ₂ Injection technique and for delivery of laboratory air in Recycling and pH Controller techniques (Must) Glass pipette used for delivery of CO ₂ gas in pH Controller technique
Test Conditions				
Aeration	Oil-free compressed laboratory air at a controlled rate of 6.5 ± 1 mL/min-L throughout test period (Must)
Vessel Size & Type	Glass aquaria or non-toxic containers; glass aquaria recommended for Recycling technique
pH	pH of each effluent concentration (i.e., 100, 50, 25...) is maintained at the pH value measure at test initiation (before any aeration is started) in each individual exposure concentration and the control (Must)
Results	LC50 not calculated if there is a non-dose related response that may be due to a gradient of pH values observed during testing across concentrations; 100% wastewater effluent sample still acceptable if other validity criteria are met

