Sublethal Toxicity Test Checklists

Environment Canada

2005 to 2016

Report Assessment Checklist for the Metal Mining EEM Program: Test of Larval Growth and Survival using Fathead minnows*

Effluent Sample Identification

Client Name/Location:_____

Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Red text reflects February 2011 second edition method changes
- Column one of the table lists reporting & method requirements (as per Schedule 5 of the Metal Mining Effluent Regulations). Reporting requirements are specified in regular type, while method "must" requirements are indicated in **bold type**.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Effluent Sample					
Effluent type identified (e.g., process effluent, final effluent)					X
Information on labelling/coding of sample					X
Measurement of temperature of the sample or of one subsample upon receipt at the laboratory					X
Date(s) for sample collection and date(s)/time(s) for sample receipt at lab					X
Date for test start (**≤ 3 d of sample collection**)					
Test Organisms					
Species (***Pimephales promelas***)					
Source (**all from the same batch**)					
Age at start of test (**≤ 24 h old larvae**)					
Any unusual appearance, behaviour, or treatment of larvae before their use in the test (**fish appearing abnormal not selected for test)**					X
Brief statement confirming inflated swimbladders (**larvae must not be used if their swimbladders are not inflated**)					
Health Criteria for Test Organisms Cultured at Laboratory					
Weekly % mortality for breeding stock, up to and including 7-d period preceding collection of eggs for test, monitored and recorded for at least 5 days of the week (**mortality < 5% of the general population being reared; mortality < 5% of the fish in individual aquaria; if mortality between 5 and 10%, holding of fish extended for at least another 7d before collection of eggs, until less than than 5% mortality in 7d is realized; if combined incidence of mortalities and diseased fish in adult breeding stock > 10% per week at any time, that stock of fish must not be used to produce test fish)**					
Test Organisms Imported from External Supplier (EC, 1999)					
Source (name and address of supplier, **all from the same source**)					
Each shipment includes statement identifying age of test organisms, date and time of shipment, # of organisms shipped					
Any unusual appearance, behaviour, or treatment of larvae by the supplier prior to shipping, or by the testing laboratory upon arrival at the testing laboratory or in the period					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
immediately preceding the test (**excessive crowding of larvae during shipment must be avoided (i.e., such that stress is minimized and oxygen deficiency prevented); test organisms must be disease free, appear healthy (not discoloured or otherwise stressed), and behave normally; test organisms recovered from disease or previous test exposure must not be used in a test**)					
Temperature and D.O. of water in which organisms are shipped just before being shipped and upon arrival at the testing laboratory (**temperature change ≤ 3° C per 24 hours during transport; D.O. maintained at ≥ 80% during transport**)					
Brief description of test organism acclimation rate and procedure at the testing laboratory (**test organisms must not be stressed during acclimation**)					
Any deviations from test-method-specific "must" requirements for culturing of test organisms, facilities and apparatus used for culturing test organisms, and culture/holding-water conditions					X
Health Criteria for Test Organisms Imported from External Supplier (EC, 1999)					
Weekly % mortality for breeding stock, up to and including 7-d period preceding collection of eggs for test, recorded for at least 5 days of the week (**mortality < 5% of the general population being reared; mortality < 5% of the fish in individual aquaria; if mortality between 5 and 10%, holding of fish extended for at least another 7d before collection of eggs, until no more than 5% mortality in 7d is realized; if mortality of adult breeding stock > 10% per week at any time, that stock of fish not be used to produce test fish**)					
Test organism mortality rates (%) upon arrival at the test laboratory and in the 24-hour period preceding a test (**mortality ≤ 10% in 24-hour period preceding the test**)					
Test Conditions and Facilities					
Test method cited (**EPS 1/RM/22, second edition February 2011**)					
Dates or days during test when subsamples or multiple samples used (i.e., days 1-2; days 3-4; days 5-6-7)					X
Date for test completion (**test ended on day 7**)					
Description of test vessels (size, shape, type of material)					X
Person performing test					
Rate of pre-aeration of solutions, if required, prior to addition of test organisms (not exceeding 100 bubbles/min per test vessel; **minimal and controlled**)					
Duration of pre-aeration (only if D.O. of test solution < 40% or > 100% upon preparation, in which case pre-aerate all solutions for **the lesser of 20 min and attaining 40% of air saturation in the highest test conc.; test initiated at this point regardless of whether D.O. of 40-100% saturation was achieved**)					
Rate/duration of aeration, if any, during exposure of test organisms (normally no aeration; not exceeding 100 bubbles/min per test vessel; **minimal and controlled**)					
Procedure, if any, for pH adjustment (recommend no adjustment if pH of test solution within 6.5 – 8.5; pH adjusted outside this range is an option or parallel test with pH adjusted solution)					X
Procedure, if any, for sample filtration or settling and decanting (recommend none) **If indigenous organisms present, filter through a sieve with 60µm mesh openings before use**)					
Procedure, if any, for hardness adjustment (recommend none)					X
Type(s) and source(s) of control/dilution water (**same water used for preparing control and test solutions; adjusted to 25 ± 1°C before use**; not supersaturated)					
Type and quantity of any chemical(s) added to control/dilution water					X
Sample / subsample adjusted to 25 ± 1°C before use					
# and conc. of test solutions (**≥ 7 test conc. plus a control**)					
Volume and depth of solution in each test vessel (**volume ≥ 250 mL**)					
# of replicates per conc. (**equal # of replicates; ≥ 3 replicated / conc. and control**)					
Second control using lab water supply to be set-up if water other than lab water supply					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
normally used to culture the breeding stock is used as control/dilution water (**was second control set up?)**					
# of organisms (**≥ 10 fish/test vessel; equal # of fish among treatments**)					
D.O. and pH of sample just before its use					X
Temperature, D.O. and pH of test solutions and controls; test solution (> 80%) renewed at 24 hr intervals for test duration (**measured at least at start (fresh solutions) and end (old solutions) of each 24 h exposure in representative conc. (control, low, med, high); temperature 25 ± 1°C)**					
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken					X
Test Endpoints and Calculations					
Swimming behaviour, # and % mortality in each replicate test vessel (including control) as noted during each 24-h observation period over the 7 days of exposure (**test is invalid if control mortality > 20%**)					
Mean (±SD) % mortality for each treatment at test end					
Combined and cumulative mean (±SD) % of control fish which either appear dead, moribund, displayed loss of equilibrium, or showed clearly atypical swimming behaviour, at each period of observation including at test end (**test is invalid if > 20%)**					
Average dry weight per surviving larvae from control treatment at test end (**test is invalid if < 250 µg)**					
Mean (±SD) biomass for each treatment including control(s) at the end of the test, as used for the ICp calculation (i.e., total weight of surviving fish divided by number of fish at test start, presumably 10)					
If receiving water used as control/dilution, then growth, mortality and swimming behaviour of fish in the lab control water was compared to that in the sample of receiving water					X
LC50 (and 95% confidence limits) for survival; indication of quantal statistic employed					
IC25 (and 95% confidence limits) for biomass; indication that regression analysis was used (ICPIN can still be used to derive an ICp if the data do not allow regression statistics); program name & citation of statistical method(s) used; details regarding any transformation of the data, more than one model tried to fit data and best fit chosen					
Endpoints generated by regression analysis are bracketed by test concentrations (i.e., extrapolation is not acceptable, except for "> 100 %")					
Data from any high test concentration(s) resulting in 0 surviving larvae for all replicates must be removed prior to regression analyses					
Outliers (if any) are identified and their removal justified					X
Results and duration of the reference toxicant test(s) (recommend that the result fall within the warning limits (± 2 SD) of the historic reference toxicant mean) **Logarithm of concentration must be used in all calculations of mean and standard deviation.**					
If in-house cultured organisms are used, reference toxicant test conducted at the same time or within 14 d of the start of the effluent test (if performed at same time the same batch of test organisms used)					
If test organisms are imported, the reference toxicant test must be conducted on the same batch of larvae at the same time as the effluent test					
The ref tox test must be conducted under the same experimental conditions (description of any deviations from test method)					
EEM Program Specific Requirements					
If applicable, statement that EC guidance document on the importation of test organisms has been followed (EC, 1999)					
Were quantitative endpoints for effluent and reference toxicant tests provided (i.e., no less than values reported)?	-	-			
Were the test endpoints bracketed by at least one test concentration (except for ">100%")?	-	-			

* Covers reporting and method requirements outlined in the test of growth and survival using larval fathead minnows, second edition published in 2011, and Schedule 5 of the Metal Mining Effluent Regulations (June 2002)

EC (1999), Environment Canada "Recommended Procedure for the Importation of Test Organisms for Sublethal Toxicity Testing", unpublished report, 22 p. [see website http://www.ec.gc.ca/eem/ for document].

Report Assessment Checklist for the Pulp and Paper and Metal Mining EEM Program: Fertilization Assay using Echinoids (Sea Urchins and Sand Dollars)*

(Revised: May 2016)

Effluent Sample Identification

Client Name/Location:_____Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Red text reflects February 2011 second edition method changes
- Column one of the table lists reporting & method requirements (including the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations)*. Reporting requirements are specified in regular type, while "must" requirements are indicated in **bold** type.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Effluent Sample					
Effluent type identified (e.g., process effluent, final effluent)					X
Information on labelling/coding of sample					X
Temperature of sample upon arrival at lab					X
Date for sample collection and date/time for sample receipt at lab					X
Date for test start **(within 3 d of sample collection**)					
Test Organisms					
Species is one of the following: *Strongylocentrotus droebachiensis, Strongylocentrotus purpuratus, Dendraster excentricus, Arbacia punctulata, Lytechinus pictus*					
Source (all adults used to provide gametes for a test be from the same batch and source)					X
Brief description of holding time and conditions for adults					X
Any unusual appearance, behaviour, or treatment of adults or gametes, before test start					X
Any deviations from test-method-specific "must" requirements for culture/holding conditions for echinoids held in lab for >3 days or for echinoids held for immediate use (≤3 d)					
Health Criteria					
Weekly % of mortalities among the adults being acclimated and held for >3 d (≤2% per day averaged over 7d preceding collection of gametes, **cumulative mortality over the same 7-d period ≤20%**)					
Percentage of mortality for adults shipped and held briefly (≤3 d) **(cumulative mortality ≤20% for 7-d period prior to shipment)**					
Test Conditions and Facilities					
Test method **(EPS 1/RM/27 and acknowledgement of February 2011 2nd Edition)** and options selected (recommend 10 min sperm + 10 min sperm & egg; options include a 20 min +20 min and a 60 min + 20 min exposure)					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Statement of test duration					X
Description of test vessels (size, shape, type of material)					X
Person performing test					X
Rate of pre-aeration of sample, if required, before starting the test (≤100 bubbles/min; **minimal and controlled**)					
Duration of pre-aeration of sample (only if D.O. is estimated to be <40% or >100% saturation in any conc., in which case pre-aerate the sample or an aliquot of sample for ≤**20 min and attaining 40% of air saturation; test initiated at this point regardless of whether D.O. of 40-100% saturation was achieved)**					
Procedure, if any, for pH adjustment of sample (recommend no adjustment if pH of test solution within 6.5-8.5; pH adjusted outside this range is an option or parallel test with pH adjusted solution)					X
Procedure, if any, for sample filtration (recommend none; **if indigenous organisms, filter through a sieve with 60 µm mesh openings before use)**					
Statement that EC guidance document (December 2001) on salinity adjustment has been followed					
Salinity 28-32 g/kg, adjusted using hypersaline brine, dry ocean salts, reagent-grade salts or deionized water					
After dry salt addition, was the stabilization (aging) period of 16 to 24 hours respected (4 ± 2 °C in darkness and in sealed container with minimum air space)?					
Type(s) and source(s) of control/dilution water **(same water used for preparing control and test solutions)**					
Type and quantity of any chemical(s) added to control/dilution water					X
Test temperature 15 ± 1°C for green sea urchin, Pacific purple sea urchins and eccentric sand dollars; 20 ± 1°C for *Arbacia* and white sea urchins					X
# and conc. of test solutions **(≥7 conc. plus control)**					
Volume and depth of solution in each test vessel (recommend 10mL; options are 5 and 2 mL)					X
# of replicates per conc. **(≥3 replicates/ conc. including controls, control has same # of replicates as for each test solution)**					
If HSB or salts added to adjust salinity, test include set of controls using same source, batch and conc.					
If uncontaminated receiving water used as control/dilution, additional control run using lab seawater previously shown to achieve valid test results					
Any test using dilution water that differs from HSB or salt control include separate set of controls prepared using this same water					
# of gametes (2000 eggs per vessel for 10 mL test volume; options include 1000 eggs for 5 mL test volume and 400 eggs for 2 mL test volume)					X
Estimated # of sperm per vessel and sperm:egg ratio					X
Sperm should represent ≥3 males and eggs ≥3 females; **perform gamete check to ensure only good quality gametes selected for test**					
If good quality gametes not available from ≥3 males and ≥3 females, fewer used if pre-test is done to determine optimal sperm:egg ratio for a given batch					
If no pre-test, gametes pooled from ≥3 males and ≥3 females as determined in gamete check					
D.O. and pH of sample just before its use					X
Temperature, salinity, D.O. and pH in aliquot of test solutions and controls at the start of the test					X
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken					X
Test Endpoints and Calculations					
# of fertilized and unfertilized eggs counted for each replicate test solution (including each control replicate) at the end of the test and mean (±SD) % fertilized eggs for					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
each test vessel (including controls) **(test is invalid if the mean fertilization rate for all replicates of controls is <60% or >98%)**					
Positive and logical dose effect curve should be obtained					X
IC25 (and 95% confidence limits) for fertilization success via non-linear regression analysis; data meets assumptions for normality and homoscedasticity; (ICPIN can be used to derive ICp if data do not allow regression statistics)					
Details regarding any weighting techniques used and indication of quantitative statistic used					
Name and citation of programs and methods used for calculating statistical endpoints					
Endpoints generated by regression analysis are bracketed by test concentrations; endpoint not extrapolated beyond highest test concentration					
Outliers (if any) are identified and their removal justified					
Results and duration of the reference toxicant test(s) (recommend that the result fall within the warning limits (± 2 SD) of the historic reference toxicant mean)					X
For adults held >3 d, reference toxicant test conducted within 14 d of effluent test or concurrently using same batch of gametes					
For adults held ≤3 d, reference toxicant test performed concurrently with effluent test					
The reference toxicant test conducted under same experimental conditions					
EEM Program Specific Requirements					
Was the test endpoint bracketed by at least 1 test conc. (except for ">100%")?					
For the Pulp and Paper EEM Program: Was report submitted within 90 d of test completion?					

* Covers reporting and method requirements outlined in the fertilization assay using echinoids (sea urchins and sand dollars), second edition published in 2011, and the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations* (June 2002).

EC (Environment Canada). "Revised Procedures for Adjusting Salinity of Effluent Samples for Marine Sublethal Toxicity Testing Conducted under Environmental Effects Monitoring (EEM) Programs", Unpublished Report, December 2001, 10 p. Method Development and Applications Section, Environmental Technol. Centre, Ottawa, ON (2001).

Report Assessment Checklist for the Pulp and Paper and Metal Mining EEM Program: Test of Larval Growth and Survival using Inland Silverside*

(Revised: May 2016)

Effluent Sample Identification

Client Name/Location:_____ Collection Approach: Single or Multiple sample(s) (circle one)
Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Column one of the table lists reporting & method requirements (including the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations*). Reporting requirements are specified in regular type, while "must" requirements are indicated in **bold** type.
- Where organisms are imported from an external supplier for use in a toxicity test, requirements (as specified in EC's guidance document on the importation of test organisms) are indicated in highlighted text type.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
Effluent Sample	**Y**	**N**	**Y**	**N**	**NA**
Effluent type (eg: process effluent, final effluent etc.)	...	...			X
Information on labelling/coding of sample	...	...			X
Temperature of sample upon arrival at lab	...	...			X
Date for sample collection and date/time for sample receipt at lab	...	...			X
Date for test start **(within 3 d of sample collection)**[1]	...	...	...	...	
Test Organisms Cultured at Testing Facility					
Species **(*Menidia beryllina*)** and source of organisms	...	...	...	...	
Age at start of test **(7 - 11 day old larvae)**	...	...	...	...	
Any unusual appearance, behaviour, or treatment of larvae, before their use in the test	...	...			X
Health Criterion ➢Recommend that organisms not be used in toxicity test if mortality preceding the test > 10% or if organisms appear to be unhealthy, discoloured or otherwise stressed	...	...			X
Test Organisms Imported from External Supplier					
Statement that EC guidance document on the importation of test organisms has been followed (September 1999)			...	...	
Species (***Menidia beryllina***) and source (name and address of test organism supplier)			...	...	
Age at start of test (**7-11 day old larvae**)			...	...	
Any unusual appearance, behaviour, or treatment of larvae by the supplier prior to shipping, or by the testing laboratory upon arrival at the testing laboratory or in the period immediately preceding the test (**excessive crowding of larvae during shipment must be avoided (i.e., such that stress is minimized and oxygen deficiency prevented); test organism must be disease free, appear healthy (not discoloured or otherwise stressed), and behave normally; test organisms recovered from disease or previous test exposure must not be used in a test**)			...	...	
Brief confirmation of inflated swimbladders and normal feeding behaviour (**larvae must not be used if they are not actively feeding and if their swimbladders are not inflated**)			...	...	
Temperature and D.O. of water in which organisms are shipped just before being shipped and upon arrival at the testing laboratory (**temperature change ≤ 3° C per 24 hours during**					

Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
transport; D.O. maintained at ≥ 4.0 mg/L during transport)……			…	…	
Brief description of test organism acclimation rate and procedure at the testing laboratory (**test organisms must not be stressed during acclimation**)……			…	…	
Any deviations from test-method-specific "must" requirements for culturing of test organisms, facilities and apparatus used for culturing test organisms, and culture/holding-water conditions……			…	…	
Health Criteria					
Recommend that organisms not be used in toxicity test if mortality preceding the test > 10% or if organisms appear to be unhealthy, discoloured or otherwise stressed……			…	…	
Test organisms mortality rates (%) upon arrival at the test laboratory and in the 24-hour period preceding a test (**mortality ≤ 10% in 24-hour period preceding the test**)……			…	…	
Test Conditions and Facilities					
Test method **(EPA-821-R-02-014, Method 1006.0; Third Edition, October 2002)**	…	…	…	…	
Test type (**static-renewal; renewal daily (≤ 24h) for test duration;** 7-day test)	…	…	…	…	
Dates or days during test when sub-samples or multiple samples used **(ie: days 1-2; days 3-4; days 5-6-7)**	…	…	…	…	
Date for test completion	…	…			X
Description of test vessels (size, shape, type of material)	…	…			X
Person performing test	…	…			X
Duration/rate of preaeration or aeration (recommend none unless D.O. < 4 mg/L; then aerate all test solutions at minimal effective rate, not exceeding 100 bubbles/min)	…	…			X
Procedures, if any, for pH adjustment and sample filtration……	…	…			X
Statement that EC guidance document on salinity adjustment has been followed			…	…	
Direct salt addition procedure for salinity adjustment of sample **(28 – 32 g/kg; as per EC guidance document on salinity adjustment-December 2001)**			…	…	
After dry salt addition, was the stabilization (aging) period of 16 to 24 hours respected (4 ± 2ºC in darkness and in sealed container with minimum air space)?			…	…	
Type(s) and source(s) of control/dilution water **(as per EC guidance document on salinity adjustment-December 2001)**			…	…	
Type and quantity of any chemical(s) added to control/dilution water	…	…			X
# and conc. of test solutions **(≥ 5 conc. plus direct salt addition control; and, if natural sea water has been used as dilution water, a second set of controls comprised of natural sea water)**……	…	…	…	…	
Volume and depth of solution in each test vessel (volume 500-750 mL)	…	…			X
# of replicates per conc. **(≥ 3 replicates for each conc. including controls)**	…	…	…	…	
# of organisms **(≥ 10 fish per test vessel,)**……	…	…	…	…	
Manner and rate of exchange of test solutions **(daily)**	…	…	…	…	
D.O. and pH of sample just before its use	…	…			X
Temperature, D.O., pH, and salinity of test solutions and controls **(measured at least at start and end (just before or immediately after renewal) of each 24h exposure period in representative conc. and controls in both the fresh and used solution; Temperature 25 ± 1 °C)**	…	…	…	…	
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken……	…	…			X
Results					
# and % of mortality in each test solution (including controls) as noted during each 24h observation period **(test is invalid if control mortality > 20%)** ……	…	…	…	…	
Average dry weight per original fish in each test vessel (in each replicate of each conc. and controls) **(test is invalid if average dry weight per surviving control larvae does not attain 0.50 mg when fish are dried and weighed immediately after the test; or 0.43 mg if fish are first preserved in 4% formalin or 70% ethanol)**	…	…	…	…	
LC50 (and 95% confidence limits) for survival; indication of quantal statistic employed	…	…	…	…	
IC25 (and 95% confidence limits) for growth; indication of quantitative statistic used	…	…	…	…	
Results and duration of the reference toxicant test(s) (recommend that the result fall within the warning limits (± 2 SD) of the historic reference toxicant mean)	…	…			X
Was a reference toxicant test conducted within 30 d of the *Menidia* test being performed on an effluent?			…	…	

Reporting & Method Requirements	Reported Data?	Met "must" Requirement?
Was it conducted under the same experimental conditions?		... ...
Was a reference toxicant test conducted on the same batch of externally provided *Menidia* used in the test performed on an effluent?.....		... ...
Was it conducted under the same experimental conditions?.....		... ...
Were the test endpoints bracketed by at least 1 test conc. (except for " > 100%")?		… …
For the Pulp and Paper EEM Program: Was report submitted within 90 d of test completion?		… …

*Covers reporting and method requirements outlined in the test of larval growth and survival using inland silverside, and the *Regulations Amending the Pulp and Paper Effluent Regulations*, and Schedule 5 of the *Metal Mining Effluent Regulations* (June 2002).

[1] For more information on test start date, please refer to Sections 7.2 and 7.4 of the Metal Mining EEM Guidance Document and Section 6.3 of the Pulp and Paper EEM Guidance Document.

Report Assessment Checklist for the Pulp and Paper and Metal Mining EEM Program: Toxicity Test using Early Life Stages of Salmonid Fish*

(Revised: April 2005)

Effluent Sample Identification

Client Name/Location:_____ Collection Approach: Single or Multiple sample(s) (circle one)
Testing Lab Name/Location:_____ Test Type: Static-renewal or Flow-through (circle one)

Instructions for Completion of Checklist

- Column one of the table lists reporting & method requirements (including the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations*). Reporting requirements are specified in regular type, while "must" requirements are indicated in **bold** type.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
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Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
Effluent Sample	**Y**	**N**	**Y**	**N**	**NA**
Effluent type (eg: process effluent, final effluent etc.)	...	...			X
Information on labelling/coding of sample	...	...			X
Temperature of sample upon arrival at lab	...	...			X
Date for sample collection and date/time for sample receipt at lab	...	...			X
Date for test start **(within 3 d of sample collection)**[1]	...	...	...	...	
Test Organisms					
Species and common name **(*Oncorhynchus mykiss* - rainbow trout)**	...	...	...	...	
Source (gametes or broodstock obtained from a single population and source)	...	...			X
# of female and male broodstock used for fertilization (**≥ 4 females; ≥ 3 males**)	...	...	...	...	
Description of procedure for checking sperm motility (**milt from ≥ 3 males held in separate vials; milt from each vial examined under microscope 100X; add small amount of water and mix; vials that contain inactive sperm not to be used for fertilization; fresh new milt to be obtained if all vials contain inactive sperm**)	...	...	...	...	
Description (including time interval) of procedure for fertilization of **gametes (dry mixing of eggs and milt for a min of 5 min and a max of 20 min**)	...	...	...	...	
Time interval from completion of fertilization until exposure of all groups of eggs to test solutions **(test start within 30 min following a period of 5 to 20 min for dry fertilization of eggs)**	...	...	...	...	
Any unusual appearance or treatment of gametes or eggs, before their use in the test	...	...			X
Test Conditions and Facilities					
Test method **(July 1998 2nd edition of EPS 1/RM/28 - E-test)**	...	...	...	...	
Dates or days during test when sub-samples or multiple samples used **(ie: days 1-2; days 3-4; days 5-6-7)**	...	...	...	...	
Date for test completion (7 d after fertilization)	...	...			X
Description of test chamber(s) and associated apparatus (eg: incubation units, pumps etc)	...	...			X
Person performing test	...	...			X
Rate of pre-aeration of sample or test solutions, if required, prior to addition of eggs (6.5 ±1 mL/min·L; **minimal and controlled**)	...	...	...	...	

Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
Duration of pre-aeration (only if D.O. of test solution < 60% or > 100% upon preparation, in which case pre-aerate sample or all test solutions for 30 min and if necessary for **the lesser of an additional period of ≤ 90 min or until 60 - 100% saturation is achieved; test initiated at this point regardless of whether D.O. of 60 - 100% saturation was achieved**)……..	...	...	...	...	
Rate of aeration (no more than 100 bubbles/min per chamber; **minimal and controlled**).....	...	...	...	...	
Duration of aeration (if static-renewal test, recommend gentle aeration throughout test; if flow-through test, aerate if necessary to maintain D.O. at 60 to 100% saturation and/or increase rate of exchange; normally aerate during test if effluent)……	...	...			X
Manner and rate of exchange of test solutions **(≥ 0.5 L/g·d; daily renewal of 80% of the old solution in static-renewal test)**.....	...	...	...	...	
Procedure, if any, for pH adjustment (recommend no adjustment if pH of test solution within 6.5 - 8.5; a second pH-adjusted test might be required/appropriate for pH beyond that range.....	...	...			X
Procedure, if any, for sample filtration (recommend no filtration; parallel test with filtered sample is an option).....	...	...			X
Type(s) and source(s) of control/dilution water (**same water used for preparing control and test solutions; adjusted to 14 ± 1°C before use**; not supersaturated).....	...	...	…	…	
Sample/subsample adjusted to 14 ± 1°C before use.....	…	…	...	...	
Type and quantity of any chemical(s) added to control/dilution water.....	...	...			X
# and conc. of test solutions **(≥ 5 conc. plus a control)**.....	...	...	...	...	
Volume and depth of solution in each test chamber.....	...	...			X
# of replicates per conc. (**equal # of replicates; ≥ 3 replicates/conc.**; 4 recommended)	...	...	...	...	
# of organisms (equal # of embryos into each test chamber; **≥ 30 / replicate, ie: ≥ 120 / conc.**)..	...	...	...	...	
D.O. and pH of sample just before its preparation and use	...	...			X
During the test, Temperature, D.O., and pH (**measured in representative concentrations at start and end of each 24-h periods in static-renewal test, or daily in flow-through test; Temperature must be 14 ± 1°C)**…….	...	...	...	...	
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken……	...	...			X
Results					
Average # and % of non-viable embryos (including unfertilized eggs) in each replicate and conc., including control at 7d **(test is invalid if mean % viable control embryos is < 70%)**……	...	...	...	...	
EC25 (and 95% confidence limits) for embryo viability; indication of quantal statistic employed…….	...	...	...	...	
Results of the E test with reference toxicant(s) (conducted concurrently with the effluent test; recommend that the result fall within the warning limits (± 2 SD) of the historic reference toxicant mean)…….	...	...			X
Test with reference toxicant conducted concurrently with E test on effluent?.....			…	…	
Was it conducted under the same experimental conditions?……			…	…	
Was the test endpoint bracketed by at least 1 test conc. (except for "<0.1% and > 100%")?			...	...	
For the Pulp and Paper Program: Was report submitted within 90 d of test completion?…			...	...	

* Covers reporting and method requirements outlined in the July 1998 2nd edition of the early life stages of salmonid fish test and the Amended *Pulp and Paper Effluent Regulations*, and Schedule 5 of the *Metal Mining Effluent Regulations* (June 2002).

[1] For more information on test start date, please refer to Sections 7.2 and 7.4 of the Metal Mining EEM Guidance Document and Section 6.3 of the Pulp and Paper EEM Guidance Document.

Report Assessment Checklist for the Pulp and Paper and Metal Mining EEM Programs: Test of Sexual Reproduction using the Red Macroalga *Champia parvula**

(Revised: April 2005)

Effluent Sample Identification

Client Name/Location:_____ Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Column one of the table lists reporting & method requirements (including the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations)*. Reporting requirements are specified in regular type, while "must" requirements are indicated in **bold** type.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
Effluent Sample	**Y**	**N**	**Y**	**N**	**NA**
Effluent type (eg: process effluent, final effluent etc.)	...	...			X
Information on labelling/coding of sample	...	...			X
Temperature of sample upon arrival at lab	...	...			X
Date for sample collection and date/time for sample receipt at lab	...	...			X
Date for test start **(within 3 d of sample collection)**[1]	...	...	...	...	
Test Organisms					
Species **(*Champia parvula*)**	...	...	...	...	
Source **(sexually mature male and female branches)**	...	...	...	...	
Any unusual appearance, behaviour, or treatment of test organisms, before their use in the test.	...	...			X
Health Criteria					
Organisms not be used in toxicity test if organisms appear to be unhealthy, discoloured or otherwise stressed preceding the test	...	...			X
Female plants should have trichogynes	...	...			X
Male plants should have sori with spermatia	...	...			X
Test Conditions and Facilities					
Test method **(EPA-821-R-02-014, Method 1009.0; Third Edition, October 2002)**	...	...	...	...	
Test type **(static; 2-day effluent exposure followed by 5 to 7-day recovery period in control medium for cystocarp development)**	...	...	...	...	
Date for test completion	...	...			X
Description of test vessels (size, shape, type of material)	...	...			X
Person performing test	...	...			X
Duration/rate of preaeration or aeration (recommend none unless D.O. < 4 mg/L; then aerate all test solutions at minimal effective rate, not exceeding 100 bubbles/min; recommend aeration during 5 - 7 day recovery period if shaker not used)	...	...			X
Procedures, if any, for pH adjustment and sample filtration	...	...			X
Statement that EC guidance document on salinity adjustment has been followed			...	...	
Direct salt addition procedure for salinity adjustment of sample **(28 – 32 g/kg; as per EC guidance document on salinity adjustment-December 2001)**			...	...	
After dry salt addition, was the stabilization (aging) period of 16 to 24 hours respected (4 ± 2ºC in darkness and in sealed containers with minimum air space)?			…	…	

Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
Type(s) and source(s) of control/dilution water **(as per EC guidance document on salinity adjustment-December 2001)**.			...	...	
Type and quantity of any chemical(s) added to control/dilution water.	...	...			X
# and conc. of test solutions **(≥ 5 conc. plus direct salt addition control; and, if natural sea water has been used as dilution water, a second set of controls comprised of natural sea water)**	...	...	...	...	
Volume and depth of solution in each test vessel **(volume ≥ 100 mL)**	...	...	...	...	
# of replicates per conc. (≥ **3 replicates for each conc. including controls)**	...	...	...	...	
# of organisms **(5 female branches and 2 male branches per test chamber)**	...	...	...	...	
D.O. and pH of sample just before its use	...	...			X
Temperature, D.O., pH, and salinity of test solutions and controls **(measured at 0h and 48h of the exposure period and at the beginning and end of the recovery period in representative conc. and controls; Temperature 23 ± 1 °C)**	...	...	...	...	
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken	…	…			X
Results					
# and % of mortality of the female plants in each test chamber (including controls) at the end of the recovery period **(test is invalid if female control mortality > 20%)**	...	...	...	...	
Mean # of cystocarps per plant in each test vessel (in each replicate of each conc. and controls) **(test is invalid if control plants average < 10 cystocarps per female plant)**	...	...	...	...	
IC25 (and 95% confidence limits) for cystocarp production; indication of quantitative statistic used	...	...	...	...	
Results and duration of the reference toxicant test(s) (recommend that the result fall within the warning limits (± 2 SD) of the historic reference toxicant mean)	...	...			X
Was a reference toxicant test conducted within 30 d of the *Champia* test being performed on an effluent?			...	...	
Was it conducted under the same experimental conditions?			…	…	
Was the test endpoint bracketed by at least 1 test conc. (except for "<0.1% and >100%")?			...	...	
For the Pulp and Paper EEM Program: Was report submitted within 90 d of test completion?			…	…	

* Covers reporting and method requirements outlined in the test of sexual reproduction using the red macroalga *Champia parvula*, and the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations* (June 2002).

[1] For more information on test start date, please refer to Sections 7.2 and 7.4 of the Metal Mining EEM Guidance Document and Section 6.3 of the Pulp and Paper EEM Guidance Document.

Report Assessment Checklist for the Pulp and Paper and Metal Mining EEM Program: Test of Larval Growth and Survival using Topsmelt*

(Revised: April 2005)

Effluent Sample Identification

Client Name/Location:_____ Collection Approach: Single or Multiple sample(s) (circle one)
Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Column one of the table lists reporting & method requirements (including the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations*). Reporting requirements are specified in regular type, while "must" requirements are indicated in **bold** type.
- Where organisms are imported from an external supplier for use in a toxicity test, requirements (as specified in EC's guidance document on the importation of test organisms) are indicated in highlighted text type.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
Effluent Sample	**Y**	**N**	**Y**	**N**	**NA**
Effluent type (eg: process effluent, final effluent etc.)	...	...			X
Information on labelling/coding of sample	...	...			X
Temperature of sample upon arrival at lab	...	...			X
Date for sample collection and date/time for sample receipt at lab	...	...			X
Date for test start **(within 3 d of sample collection)**[1]	...	...	...	...	
Test Organisms Cultured at Testing Facility					
Species (***Atherinops affinis***) and source of organisms	...	...	...	...	
Age at start of test **(9 - 15 days post-hatch)**	...	...	...	...	
Any unusual appearance, behaviour, or treatment of larvae, before their use in the test	...	...			X
Health Criterion					
• Recommend that organisms not be used in toxicity test if mortality preceding the test > 10% or if organisms appear to be unhealthy, discoloured or otherwise stressed	...	...			X
Test Organisms Imported from External Supplier					
Statement that EC guidance document on the importation of test organisms has been followed (September 1999)			...	...	
Species (*Atherinops affinis***) and source (name and address of test organism supplier)**			...	...	
Age at start of test (9-15 days post-hatch**)**			...	...	
Any unusual appearance, behaviour, or treatment of larvae by the supplier prior to shipping, or by the testing laboratory upon arrival at the testing laboratory or in the period immediately preceding the test (excessive crowding of larvae during shipment must be avoided (i.e., such that stress is minimized and oxygen deficiency prevented); test organisms must be disease free, appear healthy (not discoloured or otherwise stressed), and behave normally; test organisms recovered from disease or previous test exposure must not be used in a test)			...	...	
Brief confirmation of inflated swimbladders and normal feeding behaviour (larvae must not be used if they are not actively feeding and if their swimbladders are not inflated)			...	...	
Temperature and D.O. of water in which organisms are shipped just before being shipped and upon arrival at the testing laboratory (temperature change ≤ 3°C per 24 hours during transport; D.O. maintained at > 6.0 mg/L during transport)			...	...	

Reporting & Method Requirements	Reported Data?		Met "must" Requirement?		
Brief description of test organism acclimation rate and procedure at the testing laboratory (test organisms must not be stressed during acclimation)……			…	…	
Any deviations from test-method-specific "must" requirements for culturing of test organisms, facilities and apparatus used for culturing test organisms, and culture /holding-water conditions……			…	…	
Health Criteria					
Recommend that organisms not be used in toxicity test if mortality preceding the test > 10% or if organisms appear to be unhealthy, discoloured or otherwise stressed……			…	…	
Test organism mortality rates (%) upon arrival at the test laboratory and in the 24-hour period preceding a test (mortality ≤ 10% in 24-hour period preceding the test)……			…	…	
Test Conditions and Facilities					
Test method **(EPA/600/R-95/136, Section 11)**	…	…	…	…	
Test type **(static-renewal; renewal daily (≤ 24h) for test duration; 7-day test)**	…	…	…	…	
Dates or days during test when sub-samples or multiple samples used **(ie: days 1-2; days 3-4; days 5-6-7)**	…	…	…	…	
Date for test completion	…	…			X
Description of test vessels (size, shape, type of material)	…	…			X
Person performing test.....	…	…			X
Duration/rate of preaeration or aeration (recommend none unless D.O. < 4 mg/L; then aerate all test solutions at minimal effective rate, not exceeding 100 bubbles/min)	…	…			X
Procedures, if any, for pH adjustment and sample filtration ……	…	…			X
Statement that EC guidance document on salinity adjustment has been followed			…	…	
Direct salt addition procedure for salinity adjustment of sample **(28 – 32 g/kg; as per EC guidance document on salinity adjustment-December 2001)**.....			…	…	
After dry salt addition, was the stabilization (aging) period of 16 to 24 hours respected (4 ± 2ºC in darkness and in sealed containers with minimum air space)?.....			…	…	
Type(s) and source(s) of control/dilution water **(as per EC guidance document on salinity adjustment-December 2001)**.....			…	…	
Type and quantity of any chemical(s) added to control/dilution water.....	…	…			X
# and conc. of test solutions **(≥ 5 conc. plus direct salt addition control; and, if natural sea water has been used as dilution water, a second set of controls comprised of natural sea water)**……	…	…	…	…	
Volume and depth of solution in each test vessel **(volume 200 mL)**.....	…	…	…	…	
# of replicates per conc. **(5 replicates for each conc. including controls)**	…	…	…	…	
# of organisms **(5 fish per test vessel)** ……	…	…	…	…	
Manner and rate of exchange of test solutions **(daily)**	…	…	…	…	
D.O. and pH of sample just before its use	…	…			X
Temperature, D.O., pH and salinity of test solutions and controls **(measured at least at start and end (just before or immediately after renewal) of each 24h exposure period in representative conc. and controls in both the fresh and used solution; Temperature 20 ± 1 °C)**.....	…	…	…	…	
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken……	…	…			X
Results					
# and % of mortality in each test solution (including controls) as noted during each 24h observation period **(test is invalid if control mortality > 20%)**.....	…	…	…	…	
Average dry weight per original fish in each test vessel (in each replicate of each conc. and controls) **(test is invalid if average dry weight per surviving control larvae does not attain 0.85 mg when fish are dried and weighed immediately after the test; or 0.72 mg if fish are first preserved in 4% formalin or 70% ethanol)** ……	…	…	…	…	
LC50 (and 95% confidence limits) for survival; indication of quantal statistic employed.....	…	…	…	…	
IC25 (and 95% confidence limits) for growth; indication of quantitative statistic used……	…	…	…	…	
Results and duration of the reference toxicant test(s) **(LC50 for survival must be within the warning limits (± 2 SD) of the historic reference toxicant mean; LC50 for survival with**					

Reporting & Method Requirements	Reported Data?	Met "must" Requirement?
copper must be < 205 µg/L)	... ...	... ...
Was a reference toxicant test conducted within 30 d of the topsmelt test being performed on an effluent?.....		… …
Was it conducted under the same experimental conditions?		... ...
Was a reference toxicant test conducted on the same batch of externally supplied topsmelt used in the test performed on an effluent?.....		... ...
Was it conducted under the same experimental conditions?……		... ...
Were the test endpoints bracketed by at least 1 test conc. (except for " > 100%")?		… …
For the Pulp and Paper Program: Was report submitted within 90 d of test completion?…		… …

* Covers reporting and method requirements outlined in the test of larval growth and survival using topsmelt, and the *Regulations Amending the Pulp and Paper Effluent Regulations* and Schedule 5 of the *Metal Mining Effluent Regulations* (June 2002).

[1] For more information on test start date, please refer to Sections 7.2 and 7.4 of the Metal Mining EEM Guidance Document and Section 6.3 of the Pulp and Paper EEM Guidance Document.

Report Assessment Checklist for the Pulp & Paper and Metal Mining EEM Programs: Test of Reproduction and Survival using the Cladoceran *Ceriodaphnia dubia**

Effluent Sample Identification

Client Name/Location:_____

Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Column one of the table lists reporting & method requirements (including the Regulations Amending the Pulp and Paper Effluent Regulations and Schedule 5 of the Metal Mining Effluent Regulations). Reporting requirements are specified in regular type, while method "must" requirements are indicated in **bold type**.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Effluent Sample					
Effluent type identified (e.g., process effluent, final effluent)					X
Information on labelling/coding of sample					X
Measurement of temperature of the sample or of one subsample upon receipt at the laboratory					X
Date(s) for sample collection and date(s)/time(s) for sample receipt at lab					X
Date for test start (**≤ 3 d of sample collection**)[1]					
Test Organisms					
Species (***Ceriodaphnia dubia***)					
Source (**cultures from a single brood organism to provide test organisms**)					
Age at start of test (**< 24 h old neonates**; **all within 12 h of the same age**)					
Any unusual appearance, behaviour, or treatment of test organisms, before their use in the test					X
Health Criteria					
Mean % mortality of the brood organisms in individual cultures during 7-d period preceding test (**no more than 20% mortality**)					
Mean # of surviving young produced per adult in individual cultures during 7-d period preceding test (**average of at least 15 young per adult during their first 3 broods**)					
Neonates used to start a test must be taken from individual brood cultures containing at least 8 young produced during the 3rd or subsequent brood					
Ephippia must not be present in the culture					
Test Conditions and Facilities					
Test method (**EPS 1/RM/21, 2nd edition, February 2007**)					
Test solution renewed at ≤24 h intervals for test duration					
Dates or days during test when sub-samples or multiple samples used (**i.e., days 1-2; days 3-4; days 5-6-7**)					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Date for test completion (**when 60% of control organisms have 3 broods or within 8 days, whichever occurs first**)					
Description of test vessels (size, shape, type of material)					X
Person performing test					X
Rate of pre-aeration of solutions, if required, prior to addition of test organisms (not exceeding 100 bubbles/min per test vessel; **minimal and controlled**)					
Duration of pre-aeration (only if D.O. of test solution < 40% or > 100% upon preparation, in which case pre-aerate all solutions for **the lesser of 20 min and attaining 40% of air saturation in the highest test conc.; test initiated at this point regardless of whether D.O. of 40 - 100% saturation was achieved**)					
Rate/duration of aeration, if any, during exposure of test organisms (normally no aeration; not exceeding 100 bubbles/min per test vessel; **minimal and controlled**)					
Procedure, if any, for pH adjustment (recommend no adjustment if pH of test solution within 6.5 - 8.5; pH adjusted outside this range is an option or parallel testing with and without pH adjusted solution)					X
Procedure, if any, for sample filtration (recommend none; **if indigenous organisms, filter through a sieve with 60 µm mesh openings before use**) or hardness adjustment (recommend none)					
Type(s) and source(s) of control/dilution water (**same water used for preparing control and test solutions; adjusted to 25 ± 1°C before use**; not supersaturated)					
Type and quantity of any chemical(s) added to control/dilution water					X
Sample / subsample adjusted to 25 ± 1°C before use					
# and conc. of test solutions (**≥ 7 test conc. plus a control**)					
Volume and depth of solution in each test vessel (**volume ≥ 15 mL**)					
# of replicates per conc. (**equal # of replicates; ≥ 10 replicates/conc.**)					
Second control using culture water source set-up if water other than that in which organisms have been cultured is used as control/dilution water (**was second control set up ?**)					
# of organisms (**1 neonate/test vessel; equal # of neonates among treatments**)					
D.O. and pH of sample just before its use					X
Temperature, D.O. and pH of test solutions and controls (**measured at least at start and end (before renewal of solutions) of each 24 h exposure in representative conc.; temperature 25 ± 1°C**					
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken					X
Test Endpoints and Calculations					
Mean % mortality for first-generation daphnids in each test solution (including control) at each period of observation and test end (**test is invalid if control mortality > 20% at any time**)					
Mean # of neonates (+ SD) per first-generation daphnid in each test solution (including control), **during its first three broods only (test is invalid if 3 broods not produced in ≥ 60% of controls within 8 d and if an average of < 15 live young produced per surviving adult in the controls upon ≥ 60% of the adults achieving their 3rd brood. Note: 2 or more neonates constitutes a brood, if 1 neonate is produced it is scored as part of the neonate count for the previous or following day, any neonates produced as a 4th or subsequent brood are not included in the total # of neonates for any treatment)**					
If receiving water used as control/dilution water, mortality and reproduction rates for *C. dubia* held in 10 replicate solutions of culture water compared to those for test organisms held in the 10 replicate solutions of receiving water					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
LC50 (and 95% confidence limits) for survival; indication of quantal statistic employed					
IC25 (and 95% confidence limits) for reproduction; indication that regression analysis was used (ICPIN can still be used to derive an ICp if the data do not allow regression statistics); program name & citation of statistical method(s) used; details regarding any weighting techniques applied to the data					
Endpoints generated by regression analysis are bracketed by test concentrations (i.e., extrapolation is not acceptable)					
Outliers (if any) are identified and their removal justified					X
Results and duration of the reference toxicant test(s) (recommend that the result fall within the warning limits (± 2 SD) of the historic reference toxicant mean)					
Was a reference toxicant test conducted within 14 d before or after the *Ceriodaphnia* test on effluent was started or during it?					
Was the ref tox test conducted under the same experimental conditions?					
EEM Program Specific Requirements					
Were quantitative endpoints for effluent and reference toxicant tests provided (i.e., no less than values reported)?	-	-			
Were the test endpoints bracketed by at least one test concentration (except for ">100%")?	-	-			
For the Pulp & Paper EEM Program: was the report submitted within 90 d of test completion?					

* Covers reporting and method requirements outlined in the test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*, amended in 2007, and Regulations Amending the Pulp and Paper Effluent Regulations, and Schedule 5 of the Metal Mining Effluent Regulations (June 2002)

1 For more information on test start date, please refer to Section 7.4 of the Metal Mining EEM Guidance Document and Section 6.3 of the Pulp & Paper EEM Guidance Document.

Report Assessment Checklist for the Metal Mining EEM Program: Growth Inhibition Test using the Freshwater Macrophyte, *Lemna minor**

Effluent Sample Identification

Client Name/Location:_____

Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Column one of the table lists reporting & method requirements (including Schedule 5 of the Metal Mining Effluent Regulations). Reporting requirements are specified in regular type, while method "must" requirements are indicated in **bold type**.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Effluent Sample					
Effluent type identified (e.g., process effluent, final effluent)					X
Information on labelling/coding of sample					X
Measurement of temperature of the sample or of one subsample upon receipt at the laboratory					X
Measurement of pH before preparation and use in test					X
Date for sample collection and date/time for sample receipt at lab					X
Date for test start (**within 3 d of sample collection**)[1]					
Test Organisms					
Species (***Lemna minor*; clone identification** 8434 or 7730)					
Origin of culture (culture collection, commercial biological supplier, government, or private laboratories); **if of outside origin, cultured for ≥ 3 weeks before use**					
Sterile culture (**Axenic culture**)					
Culture medium (i.e., **must be sterile modified Hoagland's E+ medium for wastewater or receiving water tests**)					
Age of culture is **7-10 d old**; acclimation period in test medium is **18 to 24 h and ≥2 cm fresh test medium** before testing; **two, 3-frond plants** randomly transferred to each test vessel (i.e., a total of 6 fronds/vessel)					
Record any unusual appearance or treatment of known-age culture, before its use in the test					X
Health Criteria					
Frond number increased by ≥ 8-times (i.e., ≥24 fronds) in 7 days in same test medium set up as a culture test for monitoring organism health					
Test Conditions and Facilities					
Test method (**EPS 1/RM/37, 2nd edition, January 2007**)					
Type of test (static or static-renewal); solution renewal frequency (**at least every 3 d for static- renewal**)					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Date for test end and statement of duration (**7 d**)					
Name and address of test laboratory; name of person(s) performing test					X
Test vessel type (**large enough so that there is no overlapping of Lemna fronds in controls at test end**); **≥100 mL of test solution**, preferable 150 mL; covered; minimum depth of 4 cm of test solution; description of test vessel (size, shape, type of material)					
Control/Dilution Water					
Type of test medium used as control and dilution water (must be the same medium); modified APHA growth medium for testing wastewaters & receiving waters (note: SIS or Steinberg medium is used for testing chemicals), or receiving water spiked with same nutrients used in test medium (with an additional control comprised of test medium and culture dilution water)					
Type and source of water used to prepare test medium					X
Type and quantity of chemical(s) used to prepare control/dilution water					X
Test Conditions and Procedures					
≥3 replicates (i.e., 6 fronds/replicate) per concentration plus controls (for single concentration test); or ≥4 replicates per concentration plus control (for multi-concentration test with equal replicates); can have unequal replicate design with 6 reps for control, 4 replicates for lowest 3-5 test concentrations and 3 replicates for highest 4-5 test concentrations; randomised position of replicates in test area					
Number of test concentrations (**minimum ≥7 plus controls**)					
No filtration for effluents unless algae is present; procedure for filtering should be through ~1 µm glass fibre filter, then filter through 0.22 µm if necessary (same procedure to be used if receiving water is used as dilution water before nutrients are added)					
Type and quantity of nutrient added to test samples before start of test					X
Duration of pre-aeration (**20 min**) and minimum rate (e.g., 100 bubbles/min)					
Procedure, if any, for pH adjustment (no adjustment if pH of test solution is in the range 6.5 to 9.5)					X
Static test – pH: (**pH of sample at the start (Day 0) and at the end (Day 7) of test minimally for the low, medium and high test concentrations, and the control**)					
Static-renewal – pH: (**pH of sample at the start (Day 0) and at the end (Day 7) of test and before and after test solution renewal minimally for the low, medium and high test concentrations, and the control**)					
Daily temperature (**adjusted to 25 ± 2 °C before test start; no immersion heaters**). As a minimum, measured daily in representative test vessels (i.e., at least the high, medium, and low concentrations, plus the control)					
Lighting: **continuous** (fluorescent or equivalent); fluence rate: **64 to 90** µmol/m2 •s-1 at surface of test solution; within ±15% of selected light fluence rate throughout test area ; (light measured at several locations in the test area at least once during the test approximately same distance from light source as the test fronds)					
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken					X
Test Observations and Measurements					
Number and appearance of fronds in each test vessel (including control) at Day 0 and Day 7; dry weight in each vessel at Day 7					
Mean ± SD of the increase in frond number in each treatment and control(s) at test end (controls must have ≥ 8 times increase, i.e., ≥ 48 fronds)					
Mean ± SD for dry weight of fronds in each treatment and control(s) at test end					
Other observations if present (i.e., chlorosis, necrosis, appears brown or white, yellow or abnormally-sized fronds, gibbosity, colony destruction, root destruction, loss of buoyancy, other)					X

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" Requirement?		
	Y	N	Y	N	NA
Test Endpoints and Calculations					
Nominal concentrations of test solutions are corrected for the volume of nutrient stock and reported as the test concentrations (i.e., no 100%)					
Frond Number: (IC25 and 95% confidence limits); using concentrations corrected for the volume of nutrient stock; indication that regression analysis was used ((ICPIN can still be used to derive an ICp if the data do not allow regression statistics); program name and citation of statistical method(s) used; details regarding any weighting techniques applied to the data					
Frond dry Weight: (IC25 and 95% confidence limits); using concentrations corrected for the volume of nutrient stock; indication that regression analysis was used (ICPIN can still be used to derive an ICp if the data do not allow regression statistics); program name and citation of statistical method(s) used; details regarding any weighting techniques applied to the data					
Endpoints generated by regression analysis are bracketed by test concentrations (i.e., extrapolation beyond the highest test concentration is not acceptable)					
Endpoints generated by regression analysis included (i.e., no trimming) any hormetic data (i.e., growth stimulation at low concentrations followed by growth inhibition at higher test concentrations)					
Any outliers identified and the justification for their removal					X
% Stimulation: any findings of significant growth stimulation (i.e., enhanced growth at one or more higher concentrations tested or at all concentrations tested), expressed as % stimulation (frond number or dry weight), at any concentration(s)					
Results and duration of test with reference toxicant(s) with geometric mean for frond number ± 2 SD (for the same test species, clone, light, medium, vessel)					
Was a reference toxicant test started within 14 d before or after the Lemna test on effluent was started or during it?					
Was the ref tox test conducted under the same experimental conditions (same procedure and test medium)?					
Record reference toxicant used (Ni or KCl recommended)					
EEM Program Specific Requirements					
Were quantitative endpoints for effluent and ref tox tests provided (no less than values reported)?					
Were the test endpoints bracketed by at least one test concentration (except for >97%)?	-	-			

* Covers reporting and method requirements outlined in the inhibition test using the freshwater macrophyte, Lemna minor, amended in 2007 and Schedule 5 of the *Metal Mining Effluent Regulations* (June 2002).

[1] For more information on test start date, please refer to Section 7.4 of the Metal Mining EEM Guidance Document.

Report Assessment Checklist for the Pulp and Paper and Metal Mining EEM Programs: Growth Inhibition Test using a Freshwater Alga *

Effluent Sample Identification

Client Name/Location:_____

Testing Lab Name/Location:_____

Instructions for Completion of Checklist

- Column one of the table lists reporting & method requirements (including the Regulations Amending the Pulp and Paper Effluent Regulations and Schedule 5 of the Metal Mining Effluent Regulations). Reporting requirements are specified in regular type, while method "must" requirements are indicated in **bold type**.
- In column two of the table, mark under the Y if data have been reported, or under the N if data have not been provided as required by the minimum reporting of the test result.
- For column 3, this reported information can be either method minimum reporting or an EEM program requirement which is needed to evaluate whether "must" requirements have been met. If data meet the "must" requirements specified, mark under the Y in the third table column or under the N if it is not the case. Items which have no associated method or EEM "must" requirement have been hard-coded with an X under the NA.

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" requirement?		
	Y	N	Y	N	NA
Effluent Sample					
Effluent type identified (e.g., process effluent, final effluent)					X
Information on labelling/coding of sample					X
Measurement of temperature of the sample or of one subsample upon receipt at the laboratory					X
Measurement of pH before preparation and use in test					X
Date for sample collection and date/time for sample receipt at lab					X
Date for test start (**≤ 3 d of sample collection**)[1]					
Test Organisms					
Species (***Pseudokirchneriella subcapitata,* formerly *Selenastrum capricornutum)***					
Strain number and origin of culture					X
Age of known-age culture used to provide inocula of test organisms, at the start of the test **3 - 7 d old**					
Inoculum prepared ≤ 3 hrs before microplate incubation					
Initial cell density of the inoculum (**10 000 ± 1000 cells/mL**)					
Any unusual appearance or treatment of culture, before its use in the test					X
Culture Health					
Algae growth curve starting with an inoculum from the algal stock culture determined over an 8 to 10 day period using an Erlenmeyer flask (2 times per year to confirm logarithmic growth phase). Note: this is not a reporting requirement but it is a method "must".					
Test Conditions and Procedures					
Test method (**EPS 1/RM/25, 2nd edition, March 2007**)					
Date for test end and statement of duration (**72 h**)					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" requirement?		
	Y	N	Y	N	NA
Person performing test					X
Mean test temperature **(24 ± 2°C, monitored throughout the test)**					
Procedure/rate/duration of aeration of sample, if any, before start of test					X
Procedure, if any, for pH adjustment (recommend no adjustment if pH of test solution within 6.5 - 8.5; pH adjusted outside this range is an option or parallel testing with and without pH adjusted solution)					X
Final volume in each well is 220 µL					
pH of sample before any dilution, at start of test					X
pH in one standard control well at the start and one standard control well at the end of test (e.g., D6 and D7); should not differ by more than 1.5 pH units					X
Procedure for sample filtration **(through preconditioned 0.45 µm pore diameter membrane**)					
Type(s) and source(s) of control/dilution water (**same water used for preparing control and test solutions**; **all field collected dilution water is filtered through 0.45 µm filter before the addition of stock nutrients**)					
An additional control row must be set up if control/dilution water is not reagent water					
Type and quantity of any chemical(s) added to control/dilution water					
# of test concentrations (**≥ 7 plus a control**, ≥ 10 recommended. Note: less than 7 concentrations are enumerated only if : (1) cell counts of lower test concentrations show a large effect (i.e., >>IC50) has been reached, then counts at higher concentrations are not required, or (2) if cell counts show there is no effect, then only 6 concentrations are required for enumeration and the highest test concentration must be enumerated)					
# of replicates per concentration (**≥3 per test concentration and 10 for control); 8 wells are enumerated for controls and minimum 3 replicates enumerated per test concentration. If counts are inconsistent (i.e., highly variable) then the additional replicates must be counted; 2 remaining control wells are used for measuring pH**					
For metal mining effluents, the final amount of Na2EDTA•2H20 must be reduced by 25% (final concentration of 46.9 µg/L in test medium)					
If absorbance used, cell conc. (direct count) in the 3 wells containing high/medium/low test conc. and their corresponding values estimated using the absorbance method					X
Anything unusual about the test, any deviation from the test method, any problems encountered, any remedial measures taken					
Test Endpoints and Calculations					
Nominal concentrations of test solutions are corrected for the volume of nutrient stock & algal inoculum and reported as the test concentrations (i.e., no 100%)					
Cell conc. in each replicate (including control) at 72 h					
Mean cell yield (± SD) for each treatment (including control) at 72 h with corresponding CV (test is invalid if # of algal cells in control wells have not increased by a factor of > 16 in 72 h or if cell yield estimated in control wells are not homogenous (CV > 20%) or if an inhibitory gradient is detected across the control wells)					
Established relationship between cell yield and absorbance or fluorescence if these measurements are being used as a surrogate for cell yield; absorbance/fluorescence measurements are made only after cells are centrifuged and resuspended in clear solution					
IC25 (and 95% confidence limits); using concentrations corrected for the volume of algal inoculum & nutrient stock; indication that regression analysis was used (ICPIN can still be used to derive an ICp if the data do not					

Reporting & Method Requirements and EEM Requirements	Reported Data?		Met "must" requirement?		
	Y	N	Y	N	NA
allow regression statistics); program name & citation of statistical method(s) used; details regarding any weighting techniques applied to the data					
Endpoints generated by regression analysis must be bracketed by test concentrations (i.e., extrapolation beyond the highest test concentrations is not acceptable)					
Data exhibiting hormesis can be entered directly, there is no trimming of data points which show a hormetic response (i.e., growth stimulation at low concentrations followed by growth inhibition at higher test concentrations)					
If the data exhibited hormesis and ICPIN is used, control responses must be entered for those concentrations which demonstrated hormesis					
Any outliers identified and the justification for their removal					X
% Stimulation: **Any findings of significant growth stimulation (i.e., enhanced growth at one or more higher concentrations tested or at all concentrations tested), expressed as % stimulation at any concentration(s)**					
If microplate includes both a standard reagent control and a sample control (where receiving water is used as control/dilution water in the test) a statistical comparison for significant differences of means is performed					
Results of the reference toxicant test(s) (recommend that the result fall within the warning limits (± 2 SD) of the historic reference toxicant mean)					X
Was a reference toxicant test started within 14 d before or after the algae test on effluent was started or during it?					
Was the reference toxicant test conducted under the same experimental conditions as the sample test?					
Was the same batch of organisms used for tests on both the reference toxicant and the sample?					X
EEM Program Specific Requirements					
Were quantitative endpoints for effluent and reference toxicant tests provided (i.e., no less than values reported)?	-	-			
Were the test endpoints bracketed by at least one test concentration (except for >91%)?	-	-			
For the Pulp and Paper EEM Program: was the report submitted within 90 d of test completion?					

* Covers reporting and method requirements outlined in the growth inhibition test using a freshwater alga, amended in 2007, and the Amended Pulp and Paper Effluent Regulations, and Schedule 5 of the Metal Mining Effluent Regulations (June 2002).

1 For more information on test start date, please refer to Section 7.4 of the Metal Mining Guidance Document and Section 6.3 of the Pulp and Paper EEM Technical Guidance Document.