Reporting Requirements for Reference Method EPS 1/RM/13

ii. Number of control fish showing atypical/stressed behaviour
- None in Test 1, Test 2, or Test 3

iii. Mean mortality rate in solutions of effluent and control water
- Results were the same for Test 1, Test 2, and Test 3, except where noted
- Control (0%) - 0%
- 6.25% - 0%
- 12.5% - 0%
- 25% - 0%
- 50% - 0%
- 100% - 0%
- Salinity Control - 0%

iv. Estimate of 96-h LC50 in multi-concentration tests
- Results were the same for Test 1, Test 2, and Test 3
- 96hr LC_{50} concentration > 100% effluent

v. Most recent 96-h LC50 for reference toxicity test(s)
- Reference toxicity tests for Toxicant: SDS
- Test 1: (Jun-21-04) 96-h LC_{50} = 23mg/L SDS, 95% CL = 20 - 25mg/L
- Test 2: (Jul-23-04) 96-h LC_{50} = 21mg/L SDS, 95% CL = 17 - 26mg/L
- Test 3: (Aug-27-04) 96-h LC_{50} = 38mg/L SDS, 95% CL = 33 - 44mg/L

APPENDIX B

72-h Acute *Daphnia magna* Toxicity Test

Reporting Requirements for Reference Method EPS 1/RM/14

Section 8.1.1 Effluent

i. Name & location of operation generating the effluent
   - Polaris Mine, Little Cornwallis Island, Nunavut
   - Final Discharge Point for Garrow Lake is geo referenced as 75° 22' 32" N, 97° 48' 37" W.

ii. Date & time of sampling
   - Samples for monthly acute toxicity testing were collected
   - Test 1: Wednesday July 7, 2004 – 2200h
   - Test 2: Tuesday July 27, 2004 – 2130h
   - Test 3: Tuesday August 24, 2004 – 1500h

iii. Type of sample
   - Final effluent water

iv. Brief description of sampling point
   - 20m downstream of the siphon discharge point at Garrow Lake dam

v. Sampling method
   - Water was collected from at least 15cm below the surface using a water pump with silicon tubing
   - Water was collected from the upstream direction
   - The pump was flushed with site water for at least one minute prior to sample collection
   - 2 x 20L sample bottles were filled

vi. Name of person submitting samples
   - Dennis Lu (Gartner Lee), Test 1 & 2
   - Patrick Allard (Azimuth), Test 3

Section 8.1.2 Test Facilities and Conditions

i. Test type & method
   - 48-hour *Daphnia magna* LC_{50}

ii. Indications of deviations from requirements in Sections 2 to 7 of Method EPS 1/RM/13
   - No deviations from requirements
   - Salinity controls were run
   - Sample water salinity was 7ppt (Test 1); 3ppt (Test 2), and 6ppt (Test 3)

iii. Name and city of testing laboratory
   - EVS Environment Consultants, North Vancouver, BC

iv. Species of test organism
   - *Daphnia magna*

v. Date and time for start of definitive test
   - Test 1: Sunday July 11, 2004 – 1400h
   - Test 2: Friday July 30, 2004 – 1400h
   - Test 3: Friday August 27, 2004 – 1100h

vi. Person(s) performing the test and verifying the results
   - Armando Tang, Rachel DeWynter

vii. pH, temperature, dissolved oxygen, and conductivity of unadjusted, undiluted effluent
   - Test 1: pH - 7.8 – 7.9, T - 20.0 – 22.0 °C, DO – 9.1 – 11.4mg/L, C - 11850µhos/cm
   - Test 2: pH - 7.8 – 8.0, T – 21.5 – 22.0 °C, DO - 8.8 – 12.1mg/L, C - 5020µhos/cm
   - Test 3: pH – 7.8 - 8.1, T – 22.0 – 23.0 °C, DO - 8.7 – 10.2mg/L, C - 9320µmhos/cm

viii. Confirmation that no adjustment of sample or solution pH occurred
   - Test 1: No pH adjustment
   - Test 2: No pH adjustment
   - Test 3: No pH adjustment

Reporting Requirements for Reference Method EPS 1/RM/14

ix. Indication of any adjustment of hardness of effluent sample
- Test 1: No hardness adjustment
- Test 2: No hardness adjustment
- Test 3: No hardness adjustment

x. Indication of any aeration of sample
- Test 1: Aeration for 9mins
- Test 2: Gentle Aeration for 10mins
- Test 3: 20mins

xi. Concentrations and volumes tested
- For all 3 Tests the concentrations (% effluent volume / total volume) tested and total volumes used were:
  - Control (0%) - 200 mL
  - 6.25% - 200 mL
  - 12.5% - 200 mL
  - 25% - 200 mL
  - 50% - 200 mL
  - 100% - 200 mL
  - Salinity Control - 200 mL

xii. Measurements of dissolved oxygen, pH and temperature
- Test 1: DO: 8.6 – 9.1 mg/L, pH: 7.7 – 8.2, T: 20.0 - 21.5 °C
- Test 2: DO: 8.6 - 9.1 mg/L, pH: 7.6 - 8.0, T: 20.0 - 21.5 °C
- Test 3: DO: 8.5 - 9.0 mg/L, pH: 7.6 - 8.1, T: 20.5 - 22.0 °C

xiii. Estimates of time to first brood, average number of neonates per brood, and percent mortality during the seven-day period prior to the test
- Test 1: 8 days to brood, 27.5 neonates/brood, 9.5% mortality in 7d prior to test
- Test 2: 7 days to brood, 24 neonates/brood, 6.0% mortality in 7d prior to test
- Test 3: 7 days to brood, 21 neonates/brood, 0% mortality in 7d prior to test

xiv. Number of neonates per test vessel and milliliters of solution per daphnid
- Methods for all tests and dilution series were the same:
- 10 neonates per vessel
- 200 mL of solution per daphnid

Section 8.1.3 Results

i. Number of dead and/or immobile daphnids in each test solution including controls
- Results were the same for Test 1, Test 2, and Test 3, except where noted
- Control (0%) - 0 dead / immobile
- 6.25% - 0 dead / immobile
- 12.5% - 0 dead / immobile
- 25% - 0 dead / immobile
- 50% - 0 dead / immobile
- 100% - 0 dead / immobile (test 1), 3 dead/immobile (test 2), 0 dead/immobile (test 3)
- Salinity Control - 0 dead / immobile

ii. For single-concentration test the number of daphnids dead in each of three replicate effluent solutions and in each of three replicate control solutions at end of test. Also report the mean value.
- Single concentration test was not conducted, dilution series tests were conducted

Reporting Requirements for Reference Method EPS 1/RM/14

iii. Estimate of 48-h LC50 and 95% confidence limits in multi-concentration tests, 48-h EC50 for immobilization and 95% confidence limits, indication of statistical method on which results are based.

- Test 1: 48-h LC_{50} = > 100% effluent
- Test 2: 48-h LC_{50} = > 100% effluent
- Test 3: 48-h LC_{50} = > 100% effluent

iv. Most recent 48-h LC50 for reference toxicant test(s), reference chemical(s), date test initiated, historic geometric mean LC50 and warning limits.

- Reference toxicity tests for Toxicant: Zinc
- Test 1: (July-6-04) 48-h LC_{50} = 483µg/L Zinc, 95% CL = 403-578µg/L
- Test 2: (July-27-04) 48-h LC_{50} = 683µg/L Zinc, 95% CL = 578-807µg/L
- Test 3: (Aug-24-04) 48-h LC_{50} = 536µg/L Zinc, 95% CL = 438-656µg/L

APPENDIX C

7-d Topsmelt Growth and Survival Toxicity Test

Effluent Sample

i. Name & location of operation generating the effluent
   - Polaris Mine, Little Cornwallis Island, Nunavut
   - Final Discharge Point for Garrow Lake is geo referenced as 75° 22' 32" N, 97° 48' 37" W.

ii. Date & time of sampling
   - Samples for sublethal toxicity testing were collected:
     Test 1 - Wednesday July 7, 2004 – 1100h
     Test 2 - Tuesday July 27, 2004 – 0930h
     Test 3 – Tuesday Aug 24, 2004 – 1500h

iii. Type of sample
   - Final effluent water

iv. Brief description of sampling point
   - 20m downstream of the siphon discharge point at Garrow Lake dam

v. Sampling method
   - Water was collected from at least 15cm below the surface using a water pump with silicon tubing
   - Water was collected from the upstream direction
   - The pump was flushed with site water for at least one minute prior to sample collection
   - 4 x 20L sample bottles were filled

vi. Name of person submitting samples
   - Dennis Lu (Gartner Lee) Tests 1 & 2
   - Patrick Allard (Azimuth) Test 3

Test Organisms Imported from External Supplier

i. Species of test organism
   - Topsmelt (*Atherinops affinis*)

ii. Name and city of testing laboratory
   - EVS Environment Consultants, North Vancouver, BC

iii. Source of test species
   - Aquatic Bio Systems (ABS), Fort Collins, Colorado

iv. Date test species acquired on
   - Test 1 – July 8, 2004
   - Test 2 – July 29, 2004
   - Test 3 – August 26, 2004

v. Indications of deviations from EC guidance on the importation of test organisms
   - No deviations from EC requirements

vi. Percent mortality of fish in 24-hour period preceding the test
   - Test 1 - <10% mortality
   - Test 2 - <10% mortality
   - Test 3 - <10% mortality

vii. Age at start of test
   - Test 1 - 11 days post-hatch
   - Test 2 – 10 days post-hatch
   - Test 3 – 10 days post-hatch

viii. Unusual appearance, behaviour, or treatment of larvae before their use in the test
   - None noted for any test.

ix. Confirmation that larvae are actively feeding and swimbladders are not inflated
   - All tests - Larvae actively feeding and swimbladders not inflated

x. Confirmation that temperature change was <3°C and dissolved oxygen was maintained at >6mg/L during transport

- Temperature change was <2°C and dissolved oxygen supersaturated mg/L during transport

xi. Test organism acclimation rate at the testing laboratory
- Holding water conditions upon arrival were DO=supersaturated, pH = 7.9, T = 20°C
- Organisms were acclimated slowly overnight
- Addition of EVS lab seawater at intervals of 30 – 60min to reach acceptable conditions
- Organisms were acclimated to DO = 7.4mg/L, salinity = 28ppt, T=20°C

Test Facilities and Conditions

i. Test type & method
- 7-day Topsmelt (*Atherinops affinis*) Survival and Growth Toxicity Test
- Static renewal
- Sample water was renewed daily
- Reference Method - EPA/600/R-95/136 (EPAW 95-EPA West Coast)

ii. Indications of deviations from requirements in Sections 11 of Method EPA/600/R-95/136 (EPAW 95-EPA West Coast)
- No deviations from requirements
- Salinity controls were run
- Sample water salinity for
    - Test 1 was 7ppt
    - Test 2 was 2.6 ppt
    - Test 3 was 5.2ppt

iii. Date and time for start of definitive test
- Test 1 Saturday July 10, 2004 – 1730h
- Test 2 Friday July 30, 2004 – 1500h
- Test 3 Tuesday August 27, 2004 – 1230h

iv. Test vessel description
- For all tests was a 600mL beaker

v. Person(s) performing the test and verifying the results
- Test 1: Testing by: Andy Diewald, Anja Fouche, Keven Goodearle, Ann-Marie Norris and Jenny Shao; Statistical analyses by Jenny Shao and QA/QC by Julianna Kalokai
- Test 2: Testing by: Andy Diewald, Ann-Marie Norris and Jenny Shao; Statistical analysis by Jenny Shao and Kathryn Sentance; QA/QC by Armando Tang
- Test 3: Testing by Andy Diewald, Anja Fouche and Jenny Shao; Statistics by Jenny Shao and Kathryn Sentance; QA/QC by Julianna Kalokai

vi. pH, temperature, dissolved oxygen, and conductivity of unadjusted, undiluted effluent
- Test 1: pH 8.1, T 19.5 °C, DO 9.3 mg/L, C 11660 µmhos/cm
- Test 2: pH 7.8, T 20.0 °C, DO 10.7 mg/L, C 4880 µmhos/cm
- Test 3: pH 7.6, T 20.0 °C, DO 10.1 mg/L, C 9230 µmhos/cm

vii. Confirmation that no adjustment of sample or solution pH occurred
- No pH adjustment

viii. Indication of aeration of test solutions before introduction of fish
- Pre-aeration at 6.5mL/min/L for 30mins due to supersaturation of sample with O_2 when sample was heated to 19°C

ix. Indication that EC guidance document for salinity adjustment was followed
- The following was done for all 3 tests:
    - No deviations from EC guidance document on preparation of hypersaline brine
    - HSB prepared from natural seawater concentrated to 90ppt (by freezing/refreezing to remove frozen layer and concentrate salts)

- No deviations from EC guidance document for salinity adjustment of sample
- HSB was added to samples to salinity adjust them to 30ppt

x. Type and source of control/dilution water
- For all 3 tests, control/dilution water was UV-sterilized, 0.45µm-filtered natural seawater from the Vancouver Aquarium

xi. Concentrations and volumes tested:
- Concentrations (% effluent volume / total volume) tested and total volumes used were:
- For Test 1:
  - Control (0%) - 200 mL
  - Salinity Control (0%) - 200 mL
  - 4.5% - 200mL
  - 9.1% - 200mL
  - 18.2% - 200mL
  - 36.3% - 200mL
  - 72.6% - 200mL
- For Test 2:
  - Control (0%) - 200 mL
  - Salinity Control (0%) - 200 mL
  - 4.3% - 200mL
  - 8.6% - 200mL
  - 17.3% - 200mL
  - 34.5% - 200mL
  - 69.0% - 200mL
- For Test 3:
  - Control (0%) - 200 mL
  - Salinity Control (0%) - 200 mL
  - 4.4% - 200mL
  - 9.0% - 200mL
  - 18.0% - 200mL
  - 36.0% - 200mL
  - 71.0% - 200mL

xii. Number of replicated per concentration
- For all 3 tests: 5 replicates per concentration

xiii. Number of organisms added to each test vessel
- For all 3 tests: 5 fish per vessel

xiv. Manner and rate of exchange of test solutions
- For all 3 test: Daily renewal

xv. Measurements of dissolved oxygen, pH and temperature
- Test 1: DO: 6.1 - 7.9 mg/L, pH: 7.8 - 8.3, T: 19.0 - 21.0 °C
- Test 2: DO: 6.4 - 7.7 mg/L, pH: 7.8 - 8.1, T: 20.0 – 20.5 °C
- Test 3: DO: 6.4 - 7.6 mg/L, pH: 7.8 - 8.3, T: 20.0 - 21.0 °C

Results

i. Number and % of mortalities of fish in each test solution
- Test 1: Totals from all 5 replicates are presented:
  - Control (0%) – 1/25 = 4%

- Salinity Control 1/25 = 4%
- 4.5% - 0/25 = 0%
- 9.1% - 0/25 = 0%
- 18.2% - 0/25 = 0%
- 36.3% - 1/25 = 4%
- 72.6% - 2/25 = 8%

- Test 2: Totals from all 5 replicates are presented:
  - Control (0%) – 0/25 = 0%
  - Salinity Control 0/25 = 0%
  - 4.3% - 1/25 = 4%
  - 8.6% - 1/25 = 4%
  - 17.3% - 0/25 = 0%
  - 34.5% - 0/25 = 0%
  - 69.0% - 1/25 = 4%
- Test 3: Totals from all 5 replicates are presented:
  - Control (0%) – 0/25 = 0%
  - Salinity Control 0/25 = 0%
  - 4.4% - 0/25 = 0%
  - 9.0% - 0/25 = 0%
  - 18.0% - 0/25 = 0%
  - 36.0% - 1/25 = 4%
  - 71.0% - 0/25 = 0%

ii. Average dry weight per original fish in test vessel

- Test 1: Means from all 5 replicates are presented:
  - Control (0%) – 0.93 mg
  - Salinity Control – 1.08 mg
  - 4.5% - 1.00 mg
  - 9.1% - 1.04 mg
  - 18.2% - 0.96 mg
  - 36.3% - 1.10 mg
  - 72.6% - 0.99 mg
- Test 2: Means from all 5 replicates are presented:
  - Control (0%) – 0.98 mg
  - Salinity Control – 0.99 mg
  - 4.3% - 0.91 mg
  - 8.6% - 0.87 mg
  - 17.3% - 0.78 mg
  - 34.5% - 0.90 mg
  - 69.0% - 0.77 mg
- Test 3: Means from all 5 replicates are presented:
  - Control (0%) – 0.93 mg
  - Salinity Control – 0.96 mg
  - 4.4% - 1.06 mg
  - 9.0% - 0.87 mg
  - 18.0% - 0.97 mg
  - 36.0% - 0.92 mg
  - 71.0% - 0.87 mg

iii. Estimate of 7-d LC_{50} (95% CL)

- Test 1: 7-d LC_{50} concentration > 72.6% effluent (highest concentration tested due to dilution for salinity adjustment)
- Test 2: 7-d LC_{50} concentration > 69.0% effluent (highest concentration tested due to dilution for salinity adjustment)
- Test 3: : 7-d LC_{50} concentration > 71.0% effluent (highest concentration tested due to dilution for salinity adjustment)
- Quantal statistic methods not applicable

iv. Estimate of 7-d IC_{25} (95% CL) for growth

- Test 1: 7-d IC_{25} concentration > 72.6% effluent (highest concentration tested due to dilution for salinity adjustment)
- Test 2: 7-d IC_{25} concentration > 69.0% effluent (highest concentration tested due to dilution for salinity adjustment)
- Test 3: 7-d IC_{25} concentration > 71.0% effluent (highest concentration tested due to dilution for salinity adjustment)

v. Current reference toxicity tests (95% CL) for 7-d LC_{50} for survival and 7-d IC_{50} for growth

- Test 1 :Reference toxicity tests for Toxicant: Copper
  - Test conducted on July 10, 2004, same day as effluent test
  - Reference toxicant test was conducted on the same batch of externally supplied topsmelt used in the effluent test and under the same experimental conditions as the effluent test
  - 7-d LC_{50} survival = 161mg/L Cu, 95% CL = 139-188mg/L
  - 7-d IC_{50} growth = 147mg/L Cu, 95% CL = 119-169mg/L
- Test 2 :Reference toxicity tests for Toxicant: Copper
  - Test conducted on July 30, 2004, same day as effluent test
  - Reference toxicant test was conducted on the same batch of externally supplied topsmelt used in the effluent test and under the same experimental conditions as the effluent test
  - 7-d LC_{50} survival = 130mg/L Cu, 95% CL = 115-147mg/L
  - 7-d IC_{50} growth = 124mg/L Cu, 95% CL = 87-144mg/L
- Test 3 :Reference toxicity tests for Toxicant: Copper
  - Test conducted on August 27, 2004, same day as effluent test
  - Reference toxicant test was conducted on the same batch of externally supplied topsmelt used in the effluent test and under the same experimental conditions as the effluent test
  - 7-d LC_{50} survival = 121mg/L Cu, 95% CL = 107-137mg/L
  - 7-d IC_{50} growth = 128mg/L Cu, 95% CL = 85-150mg/L

vi. Reference toxicity warning limits (+/- SD) for 7-d LC_{50} for survival and 7-d IC_{50} for growth

- Test 1: Reference toxicity tests for Toxicant: Copper
  - 7-d LC_{50} survival = 137 ± 56mg/L Cu
  - 7-d IC_{50} growth = 135 ± 51mg/L Cu
- Test 2: Reference toxicity tests for Toxicant: Copper
  - 7-d LC_{50} survival = 129 ± 48mg/L Cu,
  - 7-d IC_{50} growth = 130 ± 52mg/L Cu
- Test 3: Reference toxicity tests for Toxicant: Copper
  - 7-d LC_{50} survival = 132 ± 48mg/L Cu,
  - 7-d IC_{50} growth = 131 ± 51mg/L Cu

APPENDIX D

92-h Echinoderm Fertilization Test

Reporting Requirements for Reference Method EPS1/RM/27-EC 92 (Sperm Cell)

Effluent Sample

i. Name & location of operation generating the effluent
   - Polaris Mine, Little Cornwallis Island, Nunavut
   - Final Discharge Point for Garrow Lake is geo referenced as 75° 22' 32" N, 97° 48' 37" W.

ii. Date & time of sampling
   - Samples for sublethal toxicity testing were collected:
     Test 1 - Wednesday July 7, 2004 – 1100h
     Test 2 - Tuesday July 27, 2004 – 0930h
     Test 3 – Tuesday Aug 24, 2004 – 1500h

iii. Type of sample
   - Final effluent water

iv. Brief description of sampling point
   - 20m downstream of the siphon discharge point at Garrow Lake dam

v. Sampling method
   - Water was collected from at least 15cm below the surface using a water pump with silicon tubing
   - Water was collected from the upstream direction
   - The pump was flushed with site water for at least one minute prior to sample collection
   - 4 x 20L sample bottles were filled

vi. Name of person submitting samples
   - Dennis Lu (Gartner Lee) Tests 1 & 2
   - Patrick Allard (Azimuth) Test 3

Test Organisms

i. Species of test organism
   - Sandollar Echinoid (*Dendraster excentricus*)

ii. Name and city of testing laboratory
   - EVS Environment Consultants, North Vancouver, BC

iii. Source of test species
   - M-REP, Escondido, California
   - All adults providing gametes are from the same population and source
   - Gametes are spawned in-house at EVS

iv. Date test species acquired on
   - Test 1: Test was not initiated due to inability of the Sandollars to spawn.
   - Test 2: July 30, 2004
   - Test 3: August 27, 2004

v. Holding time and conditions for adults
   - Test 1: N.A.
   - Test 2: Adults received at the testing laboratory the day of the test.
   - Test 3: Adults received at the testing laboratory the day of the test.

vi. Indications of deviations from EC guidance on the importation of test organisms
   - Test 1: na
   - Test 2: No deviations from EC requirements
   - Test 3: No deviations from EC requirements

vii. Weekly percent mortality of adults being held over 7d preceding test
   - Test 1: na
   - Test 2: <2% per day over the 7 days preceding the test
   - Test 3: <2% per day over the 7 days preceding the test

viii. Age of test organisms
   - Test 1: n.a.
   - Test 2: < 4 hours after spawning

Reporting Requirements for Reference Method EPS1/RM/27-EC 92 (Sperm Cell)

- Test 3: < 4 hours after spawning

ix. Unusual appearance, behaviour, or treatment of adults or gametes before test start
- Test 1: Test was not initiated due to inability of the Sandollars to spawn
- Test 2 Organisms appear healthy
- Test 3: Organisms appear healthy

Test Facilities and Conditions

i. Test type & method
- Echinoderm (*Dendraster excentricus*) Fertilization Toxicity Test
- Static
- Reference Method – EPS/1/RM/27 with 1997 amendments

ii. Test duration
- Test 1: na
- Test 2: 10:10 min (10min sperm + 10min sperm & egg)
- Test 3: 10:10 min (10min sperm + 10min sperm & egg)

iii. Date and time for start of definitive test
- Test 1: na
- Test 2: Friday July 30, 2004 – 1620h
- Test 3: Friday August 27, 2004 – 1334h

iv. Test vessel description
- Test 1: na
- Test 2: 16 x 125mm test tubes
- Test 3: 16 x 125mm test tubes

v. Person(s) performing the test and verifying the results
- Test 1: na
- Test 2: Testing by Kathryn Sentance and Ann-Marie Norris. Statistical analyses by Jenny Shao and Kathryn Sentance. QA/QC reviewed by Julianna Kalokai.
- Test 3: Testing by Kathryn Sentance and Ann-Marie Norris. Statistical analyses by Jenny Shao and Kathryn Sentance. QA/QC reviewed by Julianna Kalokai.

vi. Indication of rate and duration of pre-aeration of test solutions before initiation of test
- Test 1: na
- Test 2: No pre-aeration noted.
- Test 3: No pre-aeration noted.

vii. Confirmation that no adjustment of sample or solution pH occurred
- Test 1: na
- Test 2: No pH adjustment
- Test 3: No pH adjustment

viii. Procedure for sample filtration
- Test 1: na
- Test 2: No sample filtration
- Test 2: No sample filtration

ix. Procedure for preparation of hypersaline brine (HSB) as per EC guidance document on salinity adjustment – July 1997
- Test 1: na
- Test 2: 30 ppt adjusted with hypersaline brine (HSB). Preparation of HSB and salinity adjustment as per EC guidance document on Salinity adjustment – July 1997
- Test 2: 30 ppt adjusted with hypersaline brine (HSB). Preparation of HSB and salinity adjustment as per EC guidance document on Salinity adjustment – July 1997

x. Procedure for salinity adjustment as per EC guidance document on salinity adjustment – July 1997

Reporting Requirements for Reference Method EPS1/RM/27-EC 92 (Sperm Cell)

- No deviations from EC guidance for salinity adjustment
- Test 1: na
- Test 2: salinity adjusted from 2.8 to 28 ppt
- Test 3: salinity adjusted from 5 to 28 ppt

xi. Type and source of control/dilution water
- Test 1: na
- Test 2: UV-sterilized, 0.45µm-filtered natural seawater from the Vancouver Aquarium
- Test 3: UV-sterilized, 0.45µm-filtered natural seawater from the Vancouver Aquarium

xii. Concentrations and volumes tested
- Test 1: na
- Test 2: Concentrations (% effluent volume / total volume) tested and total volumes used were:
    - Control (0%) - 10mL
    - Salinity Control (0%) - 10mL
    - 4.6% - 10mL
    - 9.1% - 10mL
    - 18.3% - 10mL
    - 36.6% - 10mL
    - 73.1% - 10mL
- Test 3: Concentrations (% effluent volume / total volume) tested and total volumes used were:
    - Control (0%) - 10mL
    - Salinity Control (0%) - 10mL
    - 4.7% - 10mL
    - 9.4% - 10mL
    - 18.8% - 10mL
    - 37.5% - 10mL
    - 75.1% - 10mL

xiii. Number of replicated per concentration
- Test 1: na
- Test 2: 4 replicates per treatment concentration
- Test 3: 4 replicates per treatment concentration

xiv. Number of organisms per container
- Test 1: na
- Test 2: 100 eggs per vessel
- Test 3: 100 eggs per vessel

xv. Measurements of pH and dissolved oxygen in sample water before use
- Test 1: na
- Test 2: pH 8.1, DO 8.5
- Test 3: pH 8.0, DO 8.5

xvi. Measurements of pH, temperature, dissolved oxygen, and salinity during test
- Test 1: na
- Test 2: pH – 8.0 – 8.1, T - 15.0-16.0°C, DO - 8.2-8.5mg/L, salinity - 28ppt
- Test 3: pH – 8.2 – 8.5, T - 15.0-16.0°C, DO - 8.2-8.5mg/L, salinity - 28ppt

Results

i. Number and % of fertilized eggs in each test concentration
- Test 1: na
- Test 2: (Number is equal to percent since totals were 100)
    - Control (0%): #F = 59, 62, 56, 65 #UF = 41, 38, 44, 35
    - Salinity Control: #F = 60, 57, 59, 62 #UF = 40, 43, 41, 38

Reporting Requirements for Reference Method EPS1/RM/27-EC 92 (Sperm Cell)

- 4.6%: #F = 60, 66, 56, 58 #UF = 40, 34, 44, 42
- 9.1%: #F = 46, 42, 45, 42 #UF = 54, 58, 55, 58
- 18.3%: #F = 39, 39, 37, 36 #UF = 61, 64, 63, 64
- 36.6%: #F = 31, 34, 25, 31 #UF = 69, 66, 75, 69
- 73.1%: #F = 19, 20, 21, 20 #UF = 81, 80, 79, 80

- Test 3: (Number is equal to percent since totals were 100)
  - Control (0%): #F = 77, 80, 74, 77 #UF = 23, 20, 26, 23
  - Salinity Control: #F = 76, 73, 79, 77 #UF = 24, 27, 21, 23
  - 4.7%: #F = 78, 74, 76, 72 #UF = 22, 26, 24, 28
  - 9.4%: #F = 61, 62, 62, 60 #UF = 39, 38, 38, 40
  - 18.8%: #F = 56, 58, 59, 55 #UF = 44, 42, 41, 45
  - 37.5%: #F = 49, 50, 50, 48 #UF = 51, 50, 50, 52
  - 75.1%: #F = 25, 27, 24, 27 #UF = 75, 73, 76, 73

ii. Estimate of IC_{25} (95% CL) for fertilization success

- Test 1: na
- Test 2: IC_{25} concentration = 8.7 (7.6 – 9.9)% v/v effluent
- Test 3: IC_{25} concentration = 17.5 (11.6 – 22.6)% v/v effluent

iii. Current reference toxicity tests (95% CL) for IC_{50} for fertilization

- Test 1: na
- Test 2: Reference toxicity tests for Toxicant: Sodium Dodecyl Sulfate
  - Test conducted on July 30, 2004, same day as effluent test
  - Reference test conducted under same conditions
  - IC_{50} for fertilization = 2.1mg/L SDS, 95% CL = (1.9 – 2.4)mg/L
- Test 3: Reference toxicity tests for Toxicant: Sodium Dodecyl Sulfate
  - Test conducted on August 27, 2004, same day as effluent test
  - Reference test conducted under same conditions
  - IC_{50} for fertilization = 2.3mg/L SDS, 95% CL = (2.1 – 2.4)mg/L

iv Reference toxicant warning limits (+/- 2SD) for IC_{50} for fertilization

- Test 1: na
- Test 2: 4.2 +/- 4.8 mg/L SDS
- Test 3: 3.9 +/- 4.6 mg/L SDS

APPENDIX E

7-d Sublethal *Champia* (Algae) Toxicity Test

Reporting Requirements for Reference Method EPA/600/4-91/003 Method 1009.0

Effluent Sample

i. Name & location of operation generating the effluent
   - Polaris Mine, Little Cornwallis Island, Nunavut
   - Final Discharge Point for Garrow Lake is geo referenced as 75° 22' 32" N, 97° 48' 37" W.

ii. Date & time of sampling
   - Samples for yearly sublethal toxicity testing were collected:
     Test 1 - Wednesday July 7, 2004 – 1100h
     Test 2 - Tuesday July 27, 2004 – 0930h
     Test 3 – Tuesday Aug 24, 2004 – 1500h

iii. Type of sample
   - Final effluent water

iv. Brief description of sampling point
   - 20m downstream of the siphon discharge point at Garrow Lake dam

v. Sampling method
   - Water was collected from at least 15cm below the surface using a water pump with silicon tubing
   - Water was collected from the upstream direction
   - The pump was flushed with site water for at least one minute prior to sample collection
   - 1 x 4L sample bottles were filled

vi. Name of person submitting samples
   - Dennis Lu (Gartner Lee) Tests 1 & 2
   - Patrick Allard (Azimuth) Test 3

vii. Temperature of water upon receipt at lab
   - Test 1: Test was not conducted as sample was delayed by weather and person qualified to conduct the work at the lab left for vacation. This was discussed with Sandra Blenkinsopp at the time to provide direction.
   - Test 2: 8°C
   - Test 3: 17°C

Test Organisms

i. Species of test organism
   - Algae (*Champia parvula*)

ii. Name and city of testing laboratory
   - Saskatchewan Research Council [SRC], Saskatoon, SK

iii. Source of test species
   - Test 1: na
   - Test 2 and Test 3
     - Sexually mature male and female branches
     - Obtained from USEPA, Hatfield Marine Science Center, Newport Oregon, 1995
     - Appear in good health
     - Females have trichogynes, males have sori with spermatia

Test Facilities and Conditions

i. Test type & method
   - *Champia parvula* sexual reproduction test
   - Static, non-renewal
   - 2-day exposure, followed by 5-7 day recovery period for cystocarp development
   - Reference Method - EPA/600/4-91/003, Method 1009.0

ii. Date and time for start of definitive test
   - Test 1: na

Reporting Requirements for Reference Method EPA/600/4-91/003 Method 1009.0

- Test 2: Friday July 30, 2004 – time not noted but lab notes state tests started within 72 hrs of collection
- Test 3: Friday August 27, 2004 – time not noted but lab notes state tests started within 72 hrs of collection

iii. Test vessel description
- Test 1: na
- Test 2: 270mL transparent polystyrene cups with polystyrene lids
- Test 2: 270mL transparent polystyrene cups with polystyrene lids

iv. Person(s) performing the test and verifying the results
- Test 1: na
- Tests 2 & 3 - Mary Moody

v. Indication of pre-aeration of test solutions
- Test 1: na
- Tests 2 & 3 - No pre-aeration

vi. Confirmation that no pH adjustment of sample or solution occurred
- Test 1: na
- Tests 2 & 3: - No pH adjustment

vii. Indication that EC guidance document for salinity adjustment was followed
- Test 1: na
- Test 2:
    - No deviations from EC guidance document on preparation of hypersaline brine
    - HSB prepared from natural seawater at 90ppt
    - No deviations from EC guidance document for salinity adjustment of sample
    - Salinity adjustment: 600mL effluent + 250mL HSB + 8.5mL test nutrient solution
    - Salinity of samples adjusted from 4ppt to 30ppt
- Test 3:
    - No deviations from EC guidance document on preparation of hypersaline brine
    - HSB prepared from natural seawater at 90ppt
    - No deviations from EC guidance document for salinity adjustment of sample
    - Salinity adjustment: 600mL effluent + 230mL HSB + 10 ml test nutrient solution
    - Salinity of samples adjusted from 5ppt to 30ppt

viii. Type and source of control/dilution water
- Test 1: na
- Tests 2 & 3
    - Natural seawater collected at the Pacific Environmental Center, Environment Canada, North Vancouver, BC
    - Filtered to 0.2µm and autoclaved prior to use
    - Salinity adjusted as per EC guidance document to 30ppt with HSB from the same source

ix. Concentrations and volumes of test solutions
- Concentrations (% effluent volume / total volume) tested and total volumes used were:
- Test 1: na
- Tests 2:
    - Control (Natural Seawater) (0%) - 100mL, 4.5cm depth
    - Salinity Control Brine (0%) - 100mL, 4.5cm depth
    - 4.38% - 100mL, 4.5cm depth
    - 8.75% - 100mL, 4.5cm depth
    - 17.5% - 100mL, 4.5cm depth

Reporting Requirements for Reference Method EPA/600/4-91/003 Method 1009.0

- 35.0% - 100mL, 4.5cm depth
- 70.0% - 100mL, 4.5cm depth

- Tests 3:
  - Control (Natural Seawater) (0%) - 100mL, 4.5cm depth
  - Salinity Control Brine (0%) - 100mL, 4.5cm depth
  - 4.5% - 100mL, 4.5cm depth
  - 9.0% - 100mL, 4.5cm depth
  - 18.0% - 100mL, 4.5cm depth
  - 36.0% - 100mL, 4.5cm depth
  - 72.0% - 100mL, 4.5cm depth

x. Number of replicated per concentration
- Test 1: na
- Tests 2 & 3: 3 replicates per concentration

xi. Number of organisms per test chamber
- Test 1: na
- Tests 2 & 3: 5 female branches + 2 male branches per chamber

xii. Measurements of pH, temperature, dissolved oxygen, and salinity of sample before use
- Test 1: na
- Test 2: pH - 7.78, T – 22.0 °C, DO – 7.9mg/L, salinity - 4ppt
- Test 3: pH - 7.58, T – 23.0 °C, DO – 8.0mg/L, salinity - 5ppt

xiii. Measurements of pH, temperature, dissolved oxygen, and salinity of sample during test
- Test 1: na
- Test 2: pH – 8.20, T – 23 °C, DO – 7.9, salinity: 30ppt
- Test 3: pH – 8.33, T – 22 °C, DO – 7.9, salinity: 29ppt

Results

i. Number and % mortality of female plants after recovery in each test solution
- Totals from all 3 replicates are presented:
- Test 1: na
- Test 2:
  - Control (0%): 0 (0%) mortality
  - Salinity Control (0%): 0 (0%) mortality
  - 4.38%: 0 (0%) mortality
  - 8.75%: 0 (0%) mortality
  - 17.5%: 0 (0%) mortality
  - 35.0%: 0 (0%) mortality
  - 70.0%: 0 (0%) mortality
- Test 3:
  - Control (0%): 0 (0%) mortality
  - Salinity Control (0%): 0 (0%) mortality
  - 4.5%: 0 (0%) mortality
  - 9.0%: 0 (0%) mortality
  - 18.0%: 0 (0%) mortality
  - 36.0%: 0 (0%) mortality
  - 72.0%: 0 (0%) mortality