

AMBIENT TOXICITY TEST REPORT FORM

| GENERAL INFORMATION | | | | | | | | | |
|--|-------------------|-------------------------------------|--|--|------------------|-------------------------|---|--------------------|--------------------|
| PROJECT NAME: Mississippi River Train Derailment near Brownsville, MN Ambients | | | | LABORATORY NAME: Wisconsin State Laboratory of Hygiene | | | | | |
| | | | | REPORT NUMBER: 237493 | | | | | |
| REPORT TYPE: Original | | If amended, original report number: | | | | | | | |
| SAMPLE INFORMATION | | | | | | | | | |
| SAMPLE NO. | LAB NO. | FIELD NO. | SITE DESCRIPTION | | | | STATION NO. (SWIMS, STORET or LAT/LONG) | | |
| 1 | 237493001 | Hole # 1 | Hole # 1, Pool 8 Derailment near Brownsville, MN | | | | | | |
| 2 | 237493002 | Hole # 4 | Hole # 4, Pool 8 Derailment near Brownsville, MN | | | | | | |
| 3 | | | | | | | | | |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |
| 6 | | | | | | | | | |
| SAMPLE NO. | SAMPLE COLLECTION | | | SAMPLE TEMP. °C | | pH at LAB | HAND DELIVER? (If Yes, ≤ 4 hr?) | HOLD TIME ≤ 36 HR? | SAMPLE ACCEPTABLE? |
| | SAMPLE TYPE | SAMPLING DATES | DATE at LAB | COLLECTION | AT LAB | | | | |
| 1 | Grab | 1/27/2016 | 1/29/2016 | | 7.4 | 8.09 | No | Yes | Yes |
| 2 | Grab | 1/27/2016 | 1/29/2016 | | 7.9 | 8.07 | No | Yes | Yes |
| 3 | | | | | | | | | |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |
| 6 | | | | | | | | | |
| <i>Describe any unusual conditions during sampling that may influence test results. (see Part 6.1.2 of the Methods Manual for examples.)</i> | | | | | | | | | |
| COMMENTS: Both samples had ice evident upon arrival at laboratory. | | | | | | | | | |
| TEST INFORMATION | | | | | | | | | |
| ACUTE | | | | | CHRONIC | | | | |
| Date Test Initiated: | | 1/29/2016 | | | 1/29/2016 | | | | |
| QA/QC CONDITIONS | | | | | | | | | |
| | | | | | | ACUTE | CHRONIC | | |
| Temperatures maintained during test? (20 ± 1°C or 25 ± 1°C) | | | | | | Yes | Yes | | |
| Dissolved oxygen ≥ 4.0 mg/l throughout test? | | | | | | Yes | Yes | | |
| pH maintained within 6.0 - 9.0 s.u. throughout test? | | | | | | Yes | Yes | | |
| Concurrent or monthly reference tests within acceptable limits? | | | | | | Yes | Yes | | |
| Tests conducted in a carbon dioxide atmosphere throughout test? | | | | | | Yes | Yes | | |
| Light intensity for <i>Selenastrum</i> maintained throughout test? (4,300 ± 430 lux) | | | | | | | Yes | | |
| Were samples modified prior to testing? (ex. filtration, aeration, chem addition) | | | | | | No | No | | |
| COMMENTS: | | | | | | | | | |
| WATER CHEMISTRY | | | | | | | | | |
| (All values reported in mg/L, except pH and Conductivity) | | | | | | | | | |
| SAMPLE TYPE | SAMPLE NO. | HARDNESS | ALKALINITY | TOTAL AMMONIA | DISSOLVED OXYGEN | pH (s.u.) After Warming | Conductivity (µS) | | |
| SITES | 237493001 | 248 | 184 | 0.07 | 10.47 | 8.04 | 470 | | |
| | 237493002 | 240 | 182 | 0.07 | 10.92 | 8.04 | 472 | | |
| LAB WATER | MHW 16-14 | 92 | 58 | NA | 8.64 | 8.23 | 294 | | |
| | DC 16-09 | 196 | 318 | NA | 8.72 | 8.23 | 733 | | |
| COMMENTS: MHW= Moderately Hard Water was used as the lab control for the <i>Ceriodaphnia dubia</i> and the <i>Selenastrum</i> tests. | | | | | | | | | |
| DC = Dechlorinated Madison tap water was used as the lab control for the fathead minnow test. | | | | | | | | | |
| *Ammonia result is between the LOD (0.015 mg/L) and LOQ (0.0480 mg/L). | | | | | | | | | |
| NA = Not applicable | | | | | | | | | |

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ACUTE TEST CONTROL PERFORMANCE

LAB WATER CONTROLS

| | |
|---------------------|---------------------------|
| Fathead Minnow | <i>Ceriodaphnia dubia</i> |
| Survival \geq 90% | Survival > 90% |
| Yes | Yes |

COMMENTS:

ACUTE TEST DATA

| SPECIES | SITE DESCRIPTION | | Percent Survival By Replicate | | | | Mean Percent Survival | Statistical Significance* |
|------------------|------------------|------------------------|-------------------------------|-----|-----|-----|-----------------------|---------------------------|
| | | | 1 | 2 | 3 | 4 | | |
| Fathead Minnow | (0) 237493003 | Lab Water Control - DC | 100 | 100 | 100 | 100 | 100.0 | A |
| | (1) 237493001 | Hole # 1 | 100 | 100 | 100 | 100 | 100.0 | A |
| | (2) 237493002 | Hole #4 | 100 | 100 | 100 | 100 | 100.0 | A |
| Age of Organism: | | | | | | | | |
| 11 Days | | | | | | | | |

Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other (p -value > 0.05).

| SPECIES | SITE DESCRIPTION | | Percent Survival By Replicate | | | | Mean Percent Survival | Statistical Significance* |
|---------------------------|------------------|-------------------------|-------------------------------|-----|-----|-----|-----------------------|---------------------------|
| | | | 1 | 2 | 3 | 4 | | |
| <i>Ceriodaphnia dubia</i> | (0) 237493003 | Lab Water Control - MHW | 100 | 100 | 100 | 100 | 100.0 | A |
| | (1) 237493001 | Hole #1 | 100 | 100 | 100 | 100 | 100.0 | A |
| | (2) 237493002 | Hole #4 | 100 | 100 | 100 | 100 | 100.0 | A |
| Age of Organism: | | | | | | | | |
| < 24 Hours Old | | | | | | | | |

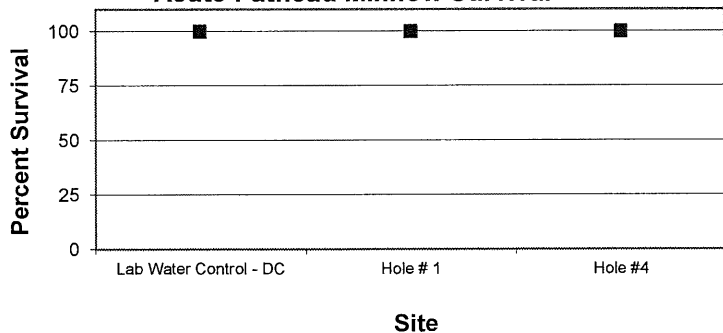
Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other (p -value > 0.05).

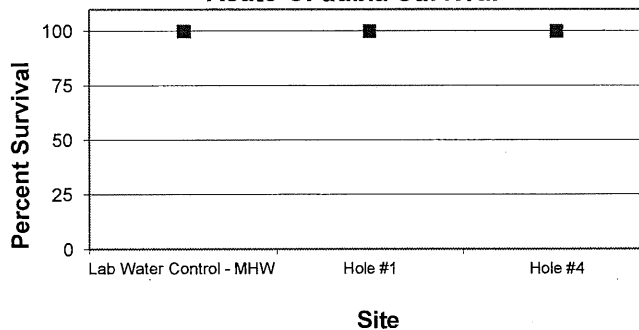
▲ = Individual Data

■ = Mean

Acute Fathead Minnow Survival



Acute C. dubia Survival



Project Name : Mississippi River Train Derailment near Brownsville, MN Ambients

Report # : 237493

Acute Test Date : 1/29/2016

CHRONIC TEST CONTROL PERFORMANCE

| LAB WATER CONTROLS | |
|---------------------------------|---|
| Fathead Minnow | <i>Ceriodaphnia dubia</i> |
| Survival ≥ 80% Yes | Survival ≥ 80% Yes |
| ≥ 0.25 mg/fish Yes | ≥ 15 neonates/female Yes |
| Survival Weight CV ≤ 40% Yes | Reproduction CV ≤ 40% Yes |
| Survival Weight % CV = 9 | Reproduction %CV = 21 ≥ 80% 3rd brood Yes |
| | ≤ 20% males Yes |

COMMENTS:

CHRONIC TEST DATA

| SPECIES | SITE DESCRIPTION | | MEAN % SURVIVAL | MEAN DRY BIOMASS PER REPLICATE PAIR (mg) | | | | | MEAN BIOMASS (mg) | Statistical Significance* |
|---------------------------------------|--------------------|-----------------|-----------------|--|-------|-------|-------|-------|-------------------|---------------------------|
| | | | | 1 | 2 | 3 | 4 | 5 | | |
| Fathead Minnow Growth & Survival Test | LC | LW Control - DC | 100 | 0.438 | 0.463 | 0.500 | 0.413 | 0.400 | 0.443 | A |
| | LW Survival Weight | | | 0.438 | 0.463 | 0.500 | 0.413 | 0.400 | | |
| | 1 | Hole # 1 | 85 | 0.475 | 0.465 | 0.388 | 0.323 | 0.408 | 0.412 | A |
| | 2 | Hole #4 | 84 | 0.488 | 0.443 | 0.410 | 0.427 | 0.393 | 0.432 | A |
| | 3 | | | | | | | | | |
| | 4 | | | | | | | | | |
| | 5 | | | | | | | | | |

Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other (p-value > 0.05). Statistical significance is based on biomass.

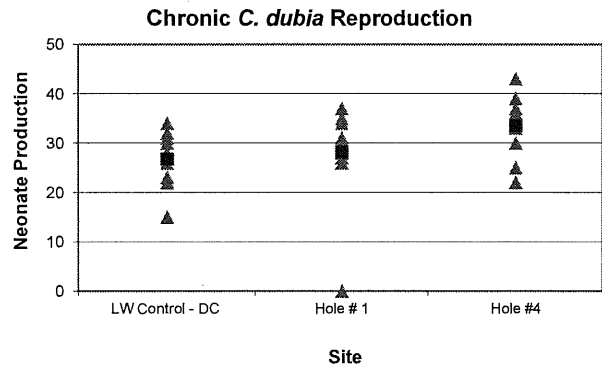
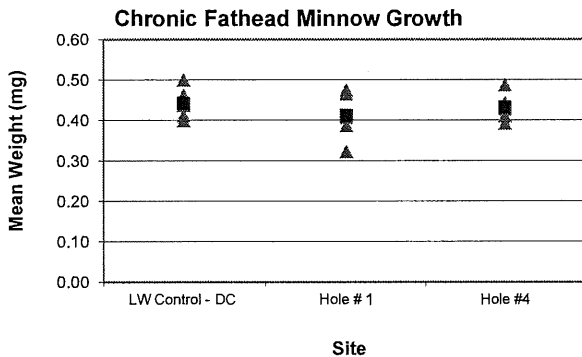
| SPECIES | SITE | NEONATE PRODUCTION BY REPLICATE | | | | | | | | | | MEAN NEONATES | % ADULT SURVIVAL | Statistical Significance* |
|---------------------------------------|-------------|---------------------------------|----|----|----|----|----|----|----|----|----|---------------|------------------|---------------------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | |
| C. dubia Reproduction & Survival Test | (LC) MHW | 26 | 30 | 32 | 27 | 15 | 34 | 22 | 23 | 31 | 28 | 27 | 90 | A |
| | (1) Hole #1 | 34 | 37 | 28 | 26 | 27 | 31 | 30 | 35 | 34 | 0 | 28 | 90 | A |
| | (2) Hole #2 | 36 | 43 | 22 | 25 | 35 | 30 | 33 | 37 | 39 | 35 | 34 | 100 | A |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

Male Production ≤ 20% Over All Treatments? Yes

Please describe any unusual behavior and/or appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

COMMENTS: * Samples with the same letter are not statistically different from each other (p-value > 0.05). Statistical significance is based on reproduction.

▲ = Individual Data ■ = Mean



CHRONIC TEST CONTROL PERFORMANCE

| | |
|-------------------------------------|--|
| LAB WATER CONTROLS | |
| <i>Selenastrum</i> | |
| ≥ 1x10 ⁶ cells/ml Yes | |
| CV ≤ 20% Yes | |
| %CV = 3 | |

GROWTH MEASUREMENT PER REPLICATE

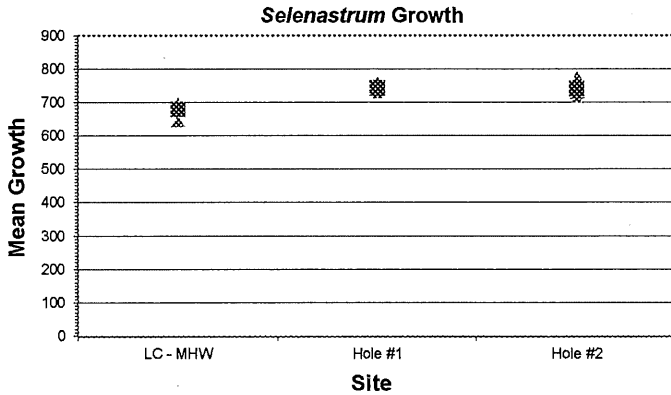
| SPECIES | SITE DESCRIPTION | | Fluorescence reading minus blank | | | | MEAN GROWTH | %CV | Statistical Significance* |
|---|------------------|----------|----------------------------------|-----|-----|-----|-------------|-----|---------------------------|
| | | | 1 | 2 | 3 | 4 | | | |
| <i>Selenastrum capricornutum</i> Growth Test | LC | LC - MHW | 647 | 684 | 700 | 690 | 680 | 3 | B |
| | 1 | Hole #1 | 755 | 758 | 732 | 730 | 744 | 2 | A |
| | 2 | Hole #2 | 775 | 731 | 716 | 743 | 741 | 3 | A |
| | 3 | | | | | | | | |
| | 4 | | | | | | | | |
| | 5 | | | | | | | | |
| | 6 | | | | | | | | |

Test Type: Microplate **Endpoint:** Fluorescence

Please describe any unusual appearance of organisms. (see Part 6.1.2 of the Methods Manual for ex.)

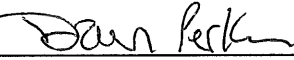
COMMENTS: * Samples with the same letter are not statistically different from each other (p-value > 0.05).

▲ = Individual Data ■ = Mean



Project Name : Mississippi River Train Derailment near Brownsville, MN Ambients
 Report # : 237493
 Chronic Test Date : 1/29/2016

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations. I also certify that these results relate only to these samples.

| | | | |
|---------------------|--|------------------|--|
| LAB REPRESENTATIVE: | Dawn Perkins | SIGNATURE: |  |
| DATE: | 2/22/2016 | | |
| PHONE: | (608) 224-6230 | WDNR LAB CERT #: | 113133790 |
| LAB ADDRESS: | Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, Madison, WI 53718 | | |
| REVIEWED BY: | Camille Danielson | DATE: | 2/22/2016 |
| PERMITTEE | NA | SIGNATURE: | NA |
| PHONE: | NA | DATE: | NA |

Send **all pages** of this form (plus any attachments or additional information which you believe to be relevant to the test) to: Biomonitoring Coordinator, Bureau of Watershed Management, Department of Natural Resources, 101 South Webster St., P.O. Box 7921, Madison, WI 53707-7921.

Copies of the State of Wisconsin Aquatic Life Toxicity Testing Methods Manual (Methods Manual) and the WET Guidance Document can be obtained from the WDNR Biomonitoring Coordinator at the address given above or at: <http://dnr.wi.gov/org/water/wm/ww/biomon/>

| TO BE COMPLETED BY THE WISCONSIN DEPARTMENT OF NATURAL RESOURCES | |
|--|-------|
| Results Entered Into Database? | |
| COMMENTS: | |
| REVIEWED BY: | DATE: |
| CC: | |
| | |
| | |

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