

**Toxicity Evaluation
of Ambient Waters from
Sheyenne River, Devils Lake,
and East Devils Lake**

Prepared by

**ASCI Corporation/ASCI Duluth
Environmental Testing Division
4444 Airpark Boulevard
Duluth, Minnesota 55811-5712**

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**US Army Corp of Engineers
USACE - St. Paul
Environmental Resources
190 5th Street E
St. Paul, Minnesota 55101-1638**

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STUDY SUMMARY

Ambient water samples collected from the Sheyenne River, Devils Lake West, Main, and East Bays, and East Devils Lake were evaluated for the presence of chronic toxicity to green algae (*Selenastrum capricornutum*), *Ceriodaphnia dubia*, and larval fathead minnows (*Pimephales promelas*) during July 8-15, 1998. U.S. EPA algal medium was used as the control water in the algal testing. Sheyenne River test concentrations were made by dilution with algal medium, and Devils Lake West Bay test concentrations were made by dilution with Sheyenne River water. Moderately hard reconstituted water (MHRW) prepared according to U.S. EPA guidelines was used as the primary control water in all fathead minnow and *Ceriodaphnia* exposures. Sheyenne River test concentrations were made by dilution with MHRW, and Devils Lake West Bay Concentrations were made by dilution with Sheyenne River water.

All control exposures met minimum control organism performance requirements defined by the U.S. EPA test methods. None of the samples tested caused significant mortality or growth inhibition of larval fathead minnow. Fathead minnow embryos exposed to the site waters did not display a significantly higher rate of terata occurrence. Additionally, embryo hatchability was not significantly reduced by exposure to any of the site waters. None of the site waters caused significant growth inhibition of green algae. All site waters, except for East Devils Lake, supported high survival and reproduction of *Ceriodaphnia*. East Devils Lake water did not support *Ceriodaphnia* survival. All organisms exposed to this site water were dead following 48 hours of exposure.

The July 1998 sodium chloride reference toxicity tests for both species met minimum performance requirements defined by the U.S. EPA, and produced IC₂₅ values within the expected ranges. Both reference tests indicated the organisms (fathead minnow and *Ceriodaphnia dubia*) were of acceptable quality and normal sensitivity.

INTRODUCTION

ASCI Corporation's Duluth Environmental Testing Division (ASCI-DETD) conducted biomonitoring tests to evaluate the toxicity of ambient water samples collected from Sheyenne River, Devils Lake West, Main, and East Bays, and East Devils Lake. The tests' purpose was to measure effects of each ambient water on green alga (*Selenastrum capricornutum*), daphnia (*Ceriodaphnia dubia* a species of zooplankton) and larval fathead minnows (*Pimephales promelas*) during short-term chronic exposures.

The green alga test endpoint was growth inhibition as measured by direct cell count via hemocytometer. Fathead minnow embryo-larval teratogenicity test endpoints were hatchability, terata (gross morphological deformities), and survival. The chronic larval fathead minnow test endpoints were survival, and growth measured as mean dry weight. *Ceriodaphnia* chronic test endpoints were survival and reproduction. The present report summarizes the results of the toxicity test battery performed July 8-15, 1998.

METHODS

The tests were conducted according to the methods described in the U.S. EPA manual, "Short-Term Methods for Measuring the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms," 1994, Third Edition, EPA/600/2-91/002. Specific testing methods were:

Green Alga, *Selenastrum capricornutum*, Growth Test Method 1003.0,

Fathead Minnow, *Pimephales promelas*, Embryo-Larval Survival and Teratogenicity Test Method 1001.0,

Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth Test Method 1000.0,
and

Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction Test Method 1002.0.

Sample Collection

All ambient water samples were grabs collected by ASCI-DETD personnel and delivered overnight to ASCI-DETD. The table below gives the sample coordinates (Global Positioning System).

| Sampling Site | GPS Coordinates |
|----------------------|--------------------------|
| Sheyenne River | N 47 53 33 W 99 23 24 |
| Devils Lake West Bay | N 5325482.96 E 485603.29 |
| Devils Lake Main Bay | N 5320215.69 E 504438.62 |
| Devils Lake East Bay | N 5317828.04 E 517710.65 |
| East Devils Lake | N 5312976 E 528999 |

Details of the sampling conditions and events are in copies of the field log (Appendix A).

The first two sample sets were shipped via UPS overnight delivery, and the third sample set was hand-delivered to the laboratory by ASCI-DETD's field crew. All samples were collected at mid-depth either by hand or using a peristaltic pump fitted with Teflon® tubing. Upon arrival, each sample was processed through ASCI-DETD Liquid Sample Receipt Registration Procedures. The sample log-in included measurement of arrival temperature, pH, conductivity, ammonia, total residual chlorine, hardness, and alkalinity.

Sample Preparation and Usage

The recommended minimum of three discreet ambient grab samples from each site was used to perform the toxicity tests. Sampling information and site descriptions are contained in the table below.

| Sample Identification | Sample Collection Dates and Times | Samples' Arrival Date | Sample Volume Gallons | Sample Usage |
|-----------------------|-----------------------------------|-----------------------|-----------------------|---------------------|
| Sheyenne River | 7/7/98 1245-1255 H | 7/8/98 | 5 | Test Days 0 & 1 |
| | 7/9/98 1240-1245 H | 7/10/98 | 5 | Test Days 2 & 3 |
| | 7/11/98 1145-1155 H | 7/12/98 | 6 | Test Days 4, 5, & 6 |
| Devils Lake West Bay | 7/7/98 1140-1200 H | 7/8/98 | 5 | Test Days 0 & 1 |
| | 7/9/98 1125-1145 H | 7/10/98 | 5 | Test Days 2 & 3 |
| | 7/11/98 0800-0820 H | 7/12/98 | 5 | Test Days 4, 5, & 6 |
| Devils Lake Main Bay | 7/7/98 1045-1055 H | 7/8/98 | 2.5 | Test Days 0 & 1 |
| | 7/9/98 0915-0925 H | 7/10/98 | 2.5 | Test Days 2 & 3 |
| | 7/11/98 0720-0730 H | 7/12/98 | 2.5 | Test Days 4, 5, & 6 |
| Devils Lake East Bay | 7/7/98 0845-0855 H | 7/8/98 | 2.5 | Test Days 0 & 1 |
| | 7/9/98 0815-0825 H | 7/10/98 | 2.5 | Test Days 2 & 3 |
| | 7/11/98 0955-1005 H | 7/12/98 | 2.5 | Test Days 4, 5, & 6 |
| East Devils Lake | 7/7/98 0700-0700 H | 7/8/98 | 2.5 | Test Days 0 & 1 |
| | 7/9/98 0645-0655 H | 7/10/98 | 2.5 | Test Days 2 & 3 |
| | 7/11/98 1400-1405 H | 7/12/98 | 2.5 | Test Days 4, 5, & 6 |

Chain of Custody forms for the sample shipments are contained in Appendix B.

Sample preparation followed testing guidance outlined by the U.S. EPA. None of the ambient samples required aeration before or during use in the toxicity tests. The aliquots of samples used in the algae tests were filtered through a 0.45 μ m glass-fiber filter to remove debris and indigenous phytoplankton. These sample aliquots were also fortified with algal medium nutrients before the test to ensure inhibition could not have been due to lack of nutrients.

A large number of indigenous invertebrates were present in the samples. The indigenous organisms were removed by sieving through a U.S. Standard #325 stainless steel mesh before the samples were used in the fathead minnow and *Ceriodaphnia* tests.

Algal Inhibition Test Performance

Sheyenne River test concentrations of 100%, 50%, and 25% were obtained by diluting the river water sample with EPA recommended algal medium (without EDTA). Devils Lake West Bay concentrations of 100%, 50%, and 25% were made by dilution with Sheyenne River Water. Devils Lake Main and East Bay, and East Devils Lake waters were all tested without dilution. All volume measurements were made using graduated cylinders of minimum size. Table 1 summarizes the test conditions employed during the 96-hour algal growth inhibition exposures.

The test solution conductivities, pH, hardness, and alkalinities were determined at test initiation. Temperature and pH were determined for one replicate from each test treatment at each 24-hour interval. At test termination, algal cell enumerations were performed via direct microscopic counting with an Improved-Neubauer[®] Hemocytometer. Algal growth was expressed as cell density in cells per ml.

Fathead Minnow Embryo-Larval Teratogenicity and Survival Test Performance

Sheyenne River test concentrations of 100%, 50%, and 25% were obtained by diluting the river water sample with MHRW. Devils Lake West Bay concentrations of 100%, 50%, and 25% were made by dilution with Sheyenne River Water. Devils Lake Main and East Bays, and East Devils Lake waters were all tested without dilution. All volume measurements were made using graduated cylinders of minimum size. These test solutions were prepared in sufficient volume to accommodate the embryo-larval test, and the fathead minnow larval and *Ceriodaphnia* chronic tests described below.

Table 2 summarizes the test conditions employed during the 7-day embryo-larval exposure. Each day test solutions were renewed. At renewal the numbers of live and dead embryos, number hatched, number of terata, and numbers of dead and live larvae were recorded. The organisms were not fed during the test period. Initial water chemistry parameters included pH, conductivity, and dissolved oxygen. Final measurements included pH and dissolved oxygen. Daily temperatures

reported are mean water bath temperatures calculated from two or three individual measurements taken each test day. The test was terminated following 7 days of exposure.

Fathead Minnow Larval Survival and Growth Test Performance

Table 3 summarizes the test conditions employed during larval fathead minnow survival and growth tests. Test concentrations were prepared as described above. The routine determinations were also the same as described above. Two times daily at a six-hour interval or three times daily at four-hour intervals the fish were fed newly hatched brine shrimp. The cysts were obtained from Biomarine, Inc., Hawthorne, California.

Each day at solution renewal the fathead minnows were observed for survival. At test termination, the fish were euthanized, placed in pre-weighed aluminum weigh boats and dried for 20 hours at 100°C. The fish were then weighed to determine growth measured as mean dry weight.

Ceriodaphnia Survival and Reproduction Test Performance

The test conditions are summarized in Table 4. The test solutions were renewed daily. Food was supplied to the organisms at solution renewal. The diet was a combination of a yeast, Cerophyl®, and fermented trout food (YCT 1800 mg/L suspended solids), and a suspension of *Selenastrum capricornutum* (3.5×10^7 cells/ml). Daily, 100 µl of both YCT and algal suspension was delivered to each test replicate.

Each day at solution renewal, the parental organisms were observed for survival and the live organisms were transferred to fresh test solution. Numbers of live, dead, and aborted offspring were counted and discarded. The test was terminated following 7 days of exposure.

Test Acceptability

The algal test is considered acceptable if the mean control algal cell count at 96 hours is $\geq 2 \times 10^5$ cells per ml. Additionally, the individual control replicate observations should not deviate by more than 20% from the mean of the control replicate values.

For the fathead minnow embryo-larval test to be acceptable, minimum control organism survival must be $\geq 80\%$. The fathead minnow larval survival and growth test is acceptable when control survival is 80% or greater and growth is 0.250 mg or greater.

Ceriodaphnia control organism survival must be $\geq 80\%$, and reproduction must be ≥ 15.0 young per female for a test to be acceptable.

Statistical Analyses

The tests' chronic data were subjected to analyses for normality and homogeneity. No observable effect concentrations (NOEC's) and lowest observable effect concentrations (LOEC's) for the data from definitive tests that proved to be normal and homogeneous were determined using Dunnett's *t*-test. Data that did not meet the normality and homogeneity assumptions were analyzed using Steel's Many One-Rank Test. Bonferroni Adjustment was used for data sets having unequal numbers of concentration replicates. Additionally, multiple comparison tests were performed comparing results from each site to all other sites. These analyses were performed using the TOXSTAT software package developed by the University of Wyoming, Laramie, Wyoming.

Inorganic Analyses

The following table summarizes the methods employed for inorganic analyses performed with each sample received.

| Parameter Measured | Method | Method Detection Limit |
|------------------------|---|------------------------|
| Bicarbonate Alkalinity | APHA 2320 B. Titration Method | 0.5 mg/L |
| Chloride | APHA 4500 D. Potentiometric Method | 1 mg/L |
| Sulfate | APHA 4500 E. Turbidimetric Method | 5 mg/L |
| Calcium | EPA 215.1 Flame Atomic Absorption Spectrometry | 0.05 mg/L |
| Magnesium | EPA Method 242.1 Flame Atomic Absorption Spectrometry | 0.02 mg/L |
| Potassium | EPA Method 258.1 Flame Atomic Absorption Spectrometry | 0.03 mg/L |
| Sodium | EPA Method 273.1 Flame Atomic Absorption Spectrometry | 0.07 mg/L |
| Total Dissolved Solids | APHA 2540 C. Oven Bake Method | 1 mg/L |

RESULTS

Arrival Chemistry Results

Table 5 contains the arrival chemistry values obtained for each sample. Sample arrival temperatures for the first sample set received on July 8, 1998, were in excess of the recommended maximum shipping temperature of 4°C. Both subsequent sample batches were received at acceptable temperatures. The arrival temperature deviation likely had little if any impact on the routine chemistry or toxicity values obtained. Total ammonia concentrations for all samples were <0.1 mg/L. Total residual chlorine concentrations were also below detection (<0.02 mg/L) for each of the samples. Sample arrival pH's were between 8.5 and 8.9 standard units. Sheyenne River conductivities were near 1200 µmhos/cm. Devils Lake West Bay waters were slightly higher in conductivity than Sheyenne River water. Main Bay water conductivities were slightly higher than the West Bay. East Bay conductivities were near 3000 µmhos/cm, and East Devils Lake conductivities were elevated to approximately 6500 µmhos/cm.

Sheyenne River total hardness values were near 300 mg/L as CaCO₃. The West Bay hardness was higher at nearly 420 mg/L. Hardness was elevated to 480 mg/L in the Main Bay, 700 mg/L in the East Bay, and 1300 mg/L in East Devils Lake. Total alkalinity was highest in Sheyenne River samples at about 560 mg/L as CaCO₃. West Bay alkalinity was approximately one half less than the river water alkalinity. Main Bay and East Bay alkalinities were near 300 mg/L and 380 mg/L, respectively. East Devils Lake had alkalinity near 500 mg/L.

East Bay and East Devils Lake conductivity values are the sole values that suggest stress to at least *Ceriodaphnia dubia*.

Routine Chemistry Results

Routine chemistry values obtained during the toxicity tests are in the raw data package. All pH, dissolved oxygen, and temperature values were within acceptable ranges as specified by the EPA methods.

Results from Algal Growth Inhibition Tests

Cell count results from the algal inhibition tests are in Table 6. The algal control medium produced an acceptable number of cells at test termination. The 96-hour control mean algal count was 136×10^4 cells per ml. None of the individual replicate values differed from the mean by more than 20% indicating variability was acceptable. One value obtained for 50% West Bay (Replicate D) was clearly an outlier as cell production was less than one-tenth of the other three replicates. This value may be due to laboratory contamination; therefore, the value was excluded from calculation of the means presented. The value was also omitted from statistical testing.

The lowest algal production was observed in the 100% concentration of the Sheyenne River water. Water from this station allowed production of 120×10^4 cells per ml. Undiluted West, Main, and East Bay waters supported algal production (250×10^4 cells/ml) that was approximately twice as

high as both the lab control and Sheyenne River water. East Devils Lake sample supported algal production of 160×10^4 cells per ml.

Appendix C contains statistical printouts from the algal data analysis. The values were both normal and homogeneous. Since the 50% West Bay D replicate was excluded from the analysis, Bonferroni Adjustment was needed. The analysis per Bonferroni t-Test showed none of the samples caused significant algal growth inhibition compared to the lab control ($p=0.05$). Samples that caused significant stimulation compared to the lab control were 25% and 50% Sheyenne River, 25% and 100% West Bay, and both Main and East Bay waters. The remaining values were statistically equal to the lab control.

Results from Fathead Minnow Embryo-Larval Teratogenicity and Survival Test

Table 7 contains the hatchability and terata results from the fathead minnow embryo-larval tests. Hatchability was determined at test day four since nearly all larvae had hatched by this point in time. Lab control water supported 88% hatch at the four-day observation. Only two samples supported less than 88% hatch by day four. Mean hatches of 87% and 84% were observed for embryos exposed to 25% Sheyenne River water and East Bay water. All other sites supported hatching of 88% or greater. The second half of the table summarizes total terata observations for the 7-day test duration. Terata occurrence in the lab control water was 5% (3 of 60 organisms exposed). Only two sites had terata occurrence greater than the control. West Bay organisms had 12% terata (7 of 60 organisms), and 14% terata (8 of 60 organisms) occurred for organisms exposed to East Bay water. Terata occurrence at all other sites ranged from 2% to 7%.

Seven-day larval survival values are in Table 8. The control organism survival was acceptable at 90%. Survival of 90% or greater occurred in the following samples; 25% Sheyenne River, 100% Sheyenne River, Main Bay, and East Devils Lake. All other site waters supported survival of 83% (East Bay) or greater.

Appendix D contains the printouts of the statistical analyses for the fathead minnow embryo-larval test. The hatchability data and the final survival data were analyzed. The hatching values were both normal and homogeneous; therefore, the analysis was performed via Dunnett's *t*-Test. None of the site waters caused a significant decrease in percent hatch at the four-day test interval ($p=0.05$).

The 7-day survival data were also normal and homogeneous. Based on survival and analyzed by Dunnett's *t*-Test, no significant reductions in larval survival occurred at any of the sites tested.

Results from Fathead Minnow Larval Survival and Growth Test

Percentage survival and mean dry weight values from the fathead minnow larval test are in Table 9. The lab control exposure supported acceptable survival (90%) and growth (0.501 mg). Both values indicated the population of fish used in the study were of acceptable quality. Sites that supported survival equal to or greater than 90% were; 50% Sheyenne River, 50% West Bay, Main Bay, and East Devils Lake. The lowest percent survival (75%) was observed for the 25% Sheyenne River Site.

Sites that produced growth greater than lab control fish growth were; 50% Sheyenne River, 25% and 100% West Bay, Main Bay, and East Bay. The lowest growth was observed for larvae exposed to 25% Sheyenne River water (0.367 mg).

Appendix D contains the statistical printouts for the larval survival and growth data. The survival values were normal and homogeneous. Results of Dunnett's *t*-Test showed none of the site waters caused statistically significant survival inhibition as compared to the lab control ($p=0.05$). The growth values were also normal and homogeneous. Again, none of the site waters resulted in statistically significant growth inhibition at $p=0.05$.

Results for *Ceriodaphnia* Survival and Reproduction Test

Table 10 summarizes the survival and reproduction results for the *Ceriodaphnia* test. The lab control organism performance was acceptable indicating the organisms selected for the study were of high quality. The control organism mean reproduction was 26.1 young per female and survival was 100%. All sites except for East Devils Lake also supported 100% survival. East Devils Lake water failed to support any *Ceriodaphnia* survival. All organisms exposed to this site water died between 24 and 48 hours of exposure.

Sites that supported reproduction in excess of lab control reproduction were; 25% and 50% Sheyenne River, 25% and 100% West Bay, and Main Bay. The remaining sites supported reproductive levels that were within 10% of the control organism reproduction.

Appendix E contains the printouts of the statistical analyses for the *Ceriodaphnia* test. The survival values did not require formal statistical analysis due to the nature of the data. Obviously, only the East Devils Lake site water caused significant lethality. Since the onset of lethality was before the 48-hour test interval, the toxicity observed can be considered to be acute in nature.

The reproduction values proved to be normal and homogeneous. Dunnett's *t*-Test results showed none of the site waters that supported survival caused significant reproductive effects compared to the lab control.

Sodium Chloride Reference Toxicity Test Results

Reference toxicity tests for fathead minnow and *Ceriodaphnia dubia* met minimum performance requirements for control survival, fish growth, and *Ceriodaphnia* reproduction. ASCI-DETD's current control chart containing fathead minnow precision data is shown in Table 11, and *Ceriodaphnia* testing precision data is shown in Table 12. Both reference toxicity tests performed with the effluent study produced IC₂₅ values within the ranges of past values. The current

coefficients of variation are 13.9% and 31.1% for fathead minnow and *Ceriodaphnia* testing methods, respectively.

Results of Major Ion Analyses

Table 13 summarizes results from the major ion analyses. The results show total dissolved solids (TDS) increase in Devils Lake from west to east. East Devils Lake waters are sufficiently high in both chloride and sulfate to cause the lethality observed for *Ceriodaphnia dubia*. However, one cannot assume from the data that TDS toxicity was the sole cause. Sheyenne River TDS values were most similar to West Bay waters. TDS values for East Devils Lake were nearly six times greater than in Sheyenne River water. Not surprisingly, all site waters had ion concentrations much higher than the lab water control calculated values. The lab water values were calculated based on the reagent-grade salts added for water preparation.

CONCLUSIONS

Algal Growth Inhibition

- None of the site waters tested caused statistically significant levels of algal growth inhibition when compared to laboratory control algal medium.
- Site waters that caused statistically significant stimulation of algal growth were; 25% and 50% Sheyenne River, 25% and 100% West Bay, and both Main and East Bay waters.

Fathead Minnow Embryo-Larval Teratogenicity and Survival

- None of the site waters caused a statistically significant decrease in fathead minnow hatchability.
- None of the site waters caused a statistically significant decrease in survival of larval fathead minnow.

Fathead Minnow Larval Survival and Growth

- None of the site waters caused a statistically significant decrease in survival of larval fathead minnow.
- None of the site waters caused statistically significant growth inhibition of larval fathead minnow.

Ceriodaphnia dubia Survival and Reproduction

- Only site water from East Devils Lake caused statistically significant lethality for *Ceriodaphnia dubia*. Complete lethality of organisms exposed to East Devils Lake water was observed by 48 hours of exposure, thereby indicating toxicity that was acute in nature.
- None of the other site waters caused statistically significant reproductive inhibition of *Ceriodaphnia dubia*.

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TABLE 1. Algal Growth Inhibition Test Conditions

| | |
|---------------------------|---|
| Organism: | <i>Selenastrum capricornutum</i> |
| Age: | 4-Day Old Culture |
| Source: | ATCC 22662 Sub-Cultured at ASCI-DETD |
| Control Water: | EPA Recommended Algal Medium without EDTA |
| Test Chamber: | 250-ml Glass Erlenmeyer® Flask |
| Volume: | 100 ml |
| Aeration: | None |
| Photoperiod: | 16 Hr Light:8 Hr Dark |
| Light Intensity: | 400 ft-candles; Supplied by Cool-White Fluorescent Bulbs |
| Incubation: | 25° ± 1°C on Rotary Shaker Table at 100 rpm. |
| Solution Preparation: | All Environmental Samples Filtered Through 0.45 µm Glass-Fiber Filter Then Nutrient Enriched Before Use |
| Test Concentrations: | Algal Medium Control, 25%, 50%, and 100% Sheyenne River; Sheyenne River Control, 25%, 50%, and 100% Devils Lake West Bay; 100% Devils Lake Main Bay; 100% Devils Lake East Bay; and 100% East Devils Lake |
| Replicates/Concentration: | 4 |
| Organisms/Replicate: | 10,000 |
| Test Endpoint: | Direct Cell Count via Hemocytometer as Measure of Growth |
| Test Acceptability: | Control Final Mean Cell Count ≥ 2 x 10 ⁵ Cells per ml, and Control Variability Should not Exceed 20% |

TABLE 2. Fathead Minnow Embryo-Larval Teratogenicity and Survival Test Conditions

| | |
|---------------------------|---|
| Organism: | Fathead Minnow (<i>Pimephales promelas</i>) |
| Age: | ≤36-Hr Old Embryos |
| Source: | ASCI-DETD Culture |
| Dilution Waters: | MHRW and Sheyenne River Water |
| Control Water: | MHRW Prepared Following Standard EPA Formula |
| Test Chamber: | 250-ml Glass Beaker |
| Volume: | 100 ml |
| Feeding: | None |
| Aeration: | None |
| Photoperiod: | 16 Hr Light:8 Hr Dark |
| Temperature: | 25° ± 1°C |
| Solution Renewal: | Daily |
| Test Concentrations: | MHRW Control, 25%, 50%, and 100% Sheyenne River; Sheyenne River Control, 25%, 50%, and 100% Devils Lake West Bay; 100% Devils Lake Main Bay; 100% Devils Lake East Bay; and 100% East Devils Lake |
| Replicates/Concentration: | 4 |
| Organisms/Replicate: | 15 |
| Organisms/Concentration: | 60 |
| Test Endpoints: | Hatchability, Terata, and Survival |
| Test Acceptability: | ≥ 80% Control Survival |

TABLE 3. Fathead Minnow Survival and Growth Test Conditions

| | |
|---------------------------|---|
| Organism: | Fathead Minnow (<i>Pimephales promelas</i>) |
| Age: | ≤ 24 Hr |
| Source: | ASCI-DETD Culture |
| Dilution Waters: | MHRW and Sheyenne River Water |
| Control Water: | MHRW Prepared Following Standard EPA Formula |
| Test Chamber: | 1-L Glass Beaker |
| Volume: | 250 ml |
| Diet: | Newly Hatched Brine Shrimp |
| Feeding: | 0.1 ml Twice per Day at a Six-Hour Interval or Three Times per Day at Four-Hour Intervals |
| Aeration: | None |
| Photoperiod: | 16 Hr Light:8 Hr Dark |
| Temperature: | 25° ± 1°C |
| Solution Renewal: | Daily |
| Test Concentrations: | MHRW Control, 25%, 50%, and 100% Sheyenne River; Sheyenne River Control, 25%, 50%, and 100% Devils Lake West Bay; 100% Devils Lake Main Bay; 100% Devils Lake East Bay; and 100% East Devils Lake |
| Replicates/Concentration: | 4 |
| Organisms/Replicate: | 10 |
| Organisms/Concentration: | 40 |
| Test Endpoints: | Survival, and Growth as Mean Dry Weight |
| Test Acceptability: | ≥ 80% Control Survival and Control Growth of ≥ 0.250 mg |

TABLE 4. *Ceriodaphnia dubia* Survival and Reproduction Test Conditions

| | |
|---------------------------|---|
| Organism: | <i>Ceriodaphnia dubia</i> |
| Age: | ≤ 8 Hr |
| Source: | ASCI-DETD Culture |
| Dilution Waters: | MHRW and Sheyenne River Water |
| Control Water: | MHRW Prepared Following Standard EPA Formula |
| Test Chamber: | 30-ml Plastic Cup |
| Volume: | 15 ml |
| Diet: | Yeast, Cerophyl®, and Fermented Trout Food Mix; and Suspension of Green Alga (<i>Selenastrum capricornutum</i>) |
| Feeding: | Fed Daily 100 µl each of YCT and Algal Suspension |
| Aeration: | None |
| Photoperiod: | 16 Hr Light:8 Hr Dark |
| Temperature: | 25° ± 1°C |
| Solution Renewal: | Daily |
| Test Concentrations: | MHRW Control, 25%, 50%, and 100% Sheyenne River; Sheyenne River Control, 25%, 50%, and 100% Devils Lake West Bay; 100% Devils Lake Main Bay; 100% Devils Lake East Bay; and 100% East Devils Lake |
| Replicates/Concentration: | 10 |
| Organisms/Replicate: | 1 |
| Organisms/Concentration: | 10 |
| Test Endpoints: | Survival and Reproduction |
| Test Acceptability: | ≥ 80% Control Survival and Control Mean Reproduction of ≥ 15 Young per Female |

TABLE 5. Routine Arrival Chemistry Values for Devils Lake Toxicity Testing Project

| Sample I.D. | Sample Collection Date (mm/dd/yy) | Sample Receipt Date (mm/dd/yy) | Arrival pH (S.U.) | Cond. (µmhos/cm) | TRC (mg/L) | Arrival Temp. (°C) | Total Amm. (mg/L) | Unionized Amm. (mg/L) | Total Hard. (mg/L as CaCO ₃) | Total Alk. (mg/L as CaCO ₃) |
|----------------------|-----------------------------------|--------------------------------|-------------------|------------------|------------|--------------------|-------------------|-----------------------|--|---|
| Shenenne River | 7/7/98 | 7/8/98 | 8.6 | 1142 | <0.02 | 9.4 | <0.1 | <0.02 | 296 | 550 |
| | 7/9/98 | 7/10/98 | 8.6 | 1231 | <0.02 | 3.0 | <0.1 | <0.02 | 294 | 558 |
| | 7/11/98 | 7/12/98 | 8.6 | 1260 | <0.02 | 4.4 | <0.1 | <0.02 | 318 | 572 |
| Devils Lake West Bay | 7/7/98 | 7/8/98 | 8.5 | 1373 | <0.02 | 11.4 | <0.1 | <0.02 | 428 | 288 |
| | 7/9/98 | 7/10/98 | 8.7 | 1467 | <0.02 | 2.8 | <0.1 | <0.02 | 422 | 284 |
| | 7/11/98 | 7/12/98 | 8.8 | 1610 | <0.02 | 2.6 | <0.1 | <0.03 | 436 | 260 |
| Devils Lake Main Bay | 7/7/98 | 7/8/98 | 8.6 | 1780 | <0.02 | 8.0 | <0.1 | <0.02 | 484 | 304 |
| | 7/9/98 | 7/10/98 | 8.7 | 1890 | <0.02 | 3.4 | <0.1 | <0.02 | 490 | 312 |
| | 7/11/98 | 7/12/98 | 8.7 | 1890 | <0.02 | 3.4 | <0.1 | <0.02 | 476 | 296 |
| Devils Lake East Bay | 7/7/98 | 7/8/98 | 8.8 | 3120 | <0.02 | 6.2 | <0.1 | <0.03 | 696 | 382 |
| | 7/9/98 | 7/10/98 | 8.8 | 3090 | <0.02 | 2.8 | <0.1 | <0.03 | 704 | 382 |
| | 7/11/98 | 7/12/98 | 8.9 | 3130 | <0.02 | 3.8 | <0.1 | <0.03 | 694 | 374 |
| East Devils Lake | 7/7/98 | 7/8/98 | 8.6 | 6320 | <0.02 | 6.2 | <0.1 | <0.02 | 1324 | 522 |
| | 7/9/98 | 7/10/98 | 8.8 | 6560 | <0.02 | 3.2 | <0.1 | <0.03 | 1294 | 512 |
| | 7/11/98 | 7/12/98 | 8.9 | 6420 | <0.02 | 3.4 | <0.1 | <0.03 | 1302 | 486 |

^a Total Ammonia Measured at 25°C and Unionized Calculations are Based on that Temperature

TABLE 6. Results of 96-Hour Algal Growth Inhibition Test with Sheyenne River, Devils Lake, and East Devils Lake Samples

| Test Solution | A | B | C | D | Mean \pm S.D. (10 ⁴) |
|---------------------------------------|-----|-----|-----|-----|---------------------------------------|
| Lab Control ^a | 123 | 163 | 118 | 140 | 136 \pm 20 |
| 25% Sheyenne River ^b | 308 | 264 | 209 | 242 | 256 \pm 42 |
| 50% Sheyenne River ^b | 264 | 231 | 198 | 209 | 226 \pm 29 |
| 100% Sheyenne River | 132 | 99 | 143 | 110 | 121 \pm 20 |
| 25% Devils Lake West Bay ^c | 231 | 220 | 220 | 231 | 226 \pm 6 |
| 50% Devils Lake West Bay ^c | 176 | 176 | 187 | 11 | 180 ^d \pm 6 |
| 100% Devils Lake West Bay | 242 | 286 | 231 | 242 | 250 \pm 24 |
| 100% Devils Lake Main Bay | 297 | 198 | 242 | 286 | 256 \pm 45 |
| 100% Devils Lake East Bay | 297 | 286 | 143 | 275 | 250 \pm 72 |
| 100% East Devils Lake | 198 | 154 | 132 | 154 | 160 \pm 28 |

^a Lab Control was USEPA Recommended Algal Medium without EDTA

^b Test Concentrations Obtained by Dilution with Algal Medium

^c Test Concentrations Obtained by Dilution with Sheyenne River Water

^d Mean Value from All Replicate Values = 138 \pm 84

