BioAnalytic Corp.
Celebrating 25 years of providing aquatic toxicity testing to business, industry and municipalities.

4 Northridge Road, DeWitt, NY 13214
Voice: 315.415.7036
Email: dogrady@twcny.rr.com
Internet: BioAnalytic.com

THE ACUTE TOXICITY OF ENVIRO TECH CHEMICAL SERVICES INC.'S

BromMax 7.1

TO THE

FRESHWATER ALGAE, SELENASTRUM CAPRICORNUTUM,

FRESHWATER CRUSTACEAN, DAPHNIA MAGNA,

AND THE

FRESHWATER FISH, PIMEPHALES PROMELAS

Report No. 1514001

January 30, 2016

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Summary of Test Results

Selenastrum capricornutum (an algal species) (OECD 201)

72 Hour EC50 (Growth Inhibition)	13.4	mg/L
No Observed Effect Concentration	3.46	mg/L

Daphnia magna (an invertebrate species) (OECD 202)

48 Hour EC50 (Immobilization)	3.83	mg/L
No Observed Effect Concentration	1.24	mg/L

Pimephales promelas (a vertebrate species) (OECD 203)

96 Hour LC50 (Death)	9.35	mg/L
No Observed Effect Concentration	3.46	mg/L

1. **INTRODUCTION**

This report presents the results of acute toxicity tests performed by BioAnalytic Corporation on behalf of Enviro Tech Chemical Services, Inc. The test article is a product referred to as "BromMax 7.1". The test results contained in this report relate to bioassays performed on the test article between December 7, 2015 and January 21, 2016. The results of three bioassays are included: (1) a 48 hour acute, static bioassay with the freshwater crustacean, *Daphnia magna*; (2) a 96 hour acute, static bioassay with the freshwater fish, *Pimephales promelas*; and (3) a 72 hour acute, static, bioassay using the freshwater algae, *Selenastrum capricornutum*.

2. **DILUTION WATER AND TEST ARTICLE**

2.1 DILUTION WATER

Dilution water used in all of the range finding and definitive bioassays was laboratory water. Characteristics of this water are presented in Table 2.1.

Table 2.1 Water Quality Characteristics of Laboratory Water used as Dilution Water in Toxicity Tests

Alkalinity	90	mg/L as $CaCO_3$
Hardness	122	mg/L as $CaCO_3$
pH	7.4	
Conductivity	280	micromho's/cm
Suspended solids	<1	mg/L
Dissolved solids	312	mg/L

2.2 TEST ARTICLE

The test article was provided by Enviro Tech Quality Control Lab. on November 30, 2015. Approximately 250mL of the test article in a plastic container was delivered via common courier. The substance was a gold liquid. The material was kept in the dark at 4°C.

The test article was readily soluble in water at all concentrations used in the study. Test concentrations were made by adding measured (mass) quantities of test article and diluting to known volumes with laboratory water. Acute endpoints were calculated on a nominal concentration basis (mg/L).

3. **TEST ORGANISMS**

3.1 Daphnia magna

Mass cultures of Daphnia magna are maintained in-house. The test animals are cultured at approximately 24 ° C in laboratory water and are fed a unicellular green alga, Selenastrum capricornutum, and a digested combination of yeast, cereal leaves, and trout chow (YCT) daily. The light regimen is 16 hours light and 8 hours dark under wide spectrum fluorescent bulbs.

Organisms used in the definitive test were less than 24 hour old neonates spawned from adults held in laboratory water.

3.2 Pimephales promelas

The fathead minnows used in the toxicity test were cultured in-house at BioAnalytic. The animals were cultured at approximately 24 °C in laboratory water and are fed live Artemia naupali and salmon starter. The light regimen is 16 hours light and 8 hours dark under wide spectrum fluorescent bulbs. Test organisms were obtained from adults spawned in laboratory water.

Organisms used in the test were between 8 and 9 days old.

Organisms were not fed during the test.

3.3 Selenastrum capricornutum

Mass cultures of Selenastrum are maintained in house at BioAnalytic. The algae are cultured in growth media at

approximately 24 °C under continuous illumination provided by wide spectrum fluorescent bulbs.

4. **TEST METHODS**

4.1 Daphnia magna Acute Test

The method followed for this toxicity test, the *Daphnia sp.* Acute Immobilisation Test, was that described in OECD Guideline for Testing of Chemicals - 202 (OECD, 1984).

Two acute endpoints were determined. The first was immobilization, and the median effected concentration (EC_{50}) after 48 hours was determined. The second acute endpoint was the concentration of test article that resulted in no observed immobilization (NOEC) also determined after 48 hours of exposure to the test article.

The test consisted of six treatments: five test article concentrations, and one laboratory water control. Four replicates of each treatment with five test organisms in each replicate were used. Test chambers were 50 mL borosilicate glass beakers each filled with 30 mL of test solution. The toxicity test was conducted in an incubator under wide spectrum fluorescent lighting. Test temperature ranged from 19.3 to 20.7 °C (mean value- 19.8 °C). The pH of test solutions ranged 7.5 in the lowest test concentration to 7.8 in the highest. All solutions

maintained their pH throughout the test (+/- 0.5). Dissolved oxygen levels maintained >80% saturation.

Test concentrations ranged from 1.24 mg/L to 9.60 mg/L with a factor of 0.6 from one concentration to the next lower concentration. EC50 values were calculated using the Spearman-Karber statistical method (Stephan, 1977).

4.2 Pimephales promelas Acute Test

The method followed for this toxicity test was "Determination of the acute lethal toxicity of substances to a freshwater fish" (ISO 7346-2, 2nd edition, 1996).

Two acute endpoints were determined; the 96 hour median lethal concentration (LC_{50}) and the acute no observed effect concentration (NOEC).

The test consisted of six treatments: five test article concentrations, and one control. Four replicates of each treatment with five test organisms in each replicate were used. Test chambers were 250 mL borosilicate glass beakers each filled with 100 mL of test solution. The toxicity test was conducted in an incubator under wide spectrum fluorescent lighting. Test temperature ranged from 24.3 to 25.5 °C (mean value- 24.8 °C). The pH of test solutions ranged 7.5 in the lowest test concentration

to 7.9 in the highest. Dissolved oxygen levels maintained >80% saturation in all test treatments.

Test article concentrations ranged from 2.07 mg/L to 16.0 mg/L with a factor of 0.6 from one concentration to the next lower concentration. The LC50 value was calculated using the Spearman-Karber statistical method (Stephan, 1977).

4.3 Algal Growth Inhibition Test

The method followed for this toxicity test, the Algal Growth Inhibition Test, is that described in OECD Guideline for Testing of Chemicals - 201 (OECD, 1984).

Two acute endpoints were determined; the 72 hour EC50 and NOEC values. The test consisted of six treatments: five test article concentrations, and one control. Three replicates of each treatment were used. Test chambers were 250 mL borosilicate glass flasks each filled with 100 mL of test solution. The toxicity test was conducted in an incubator under wide spectrum fluorescent lighting. Test chambers were stirred continuously during the test. Temperature ranged from 22.5 to 23.8 $^{\circ}$C (mean value-23.2°C). The adjusted pH of test solutions maintained 7.4 (+/- 1).

Test article concentrations ranged from 2.07 mg/L to 16.0 mg/L with a factor of 0.6 from one concentration to the next lower

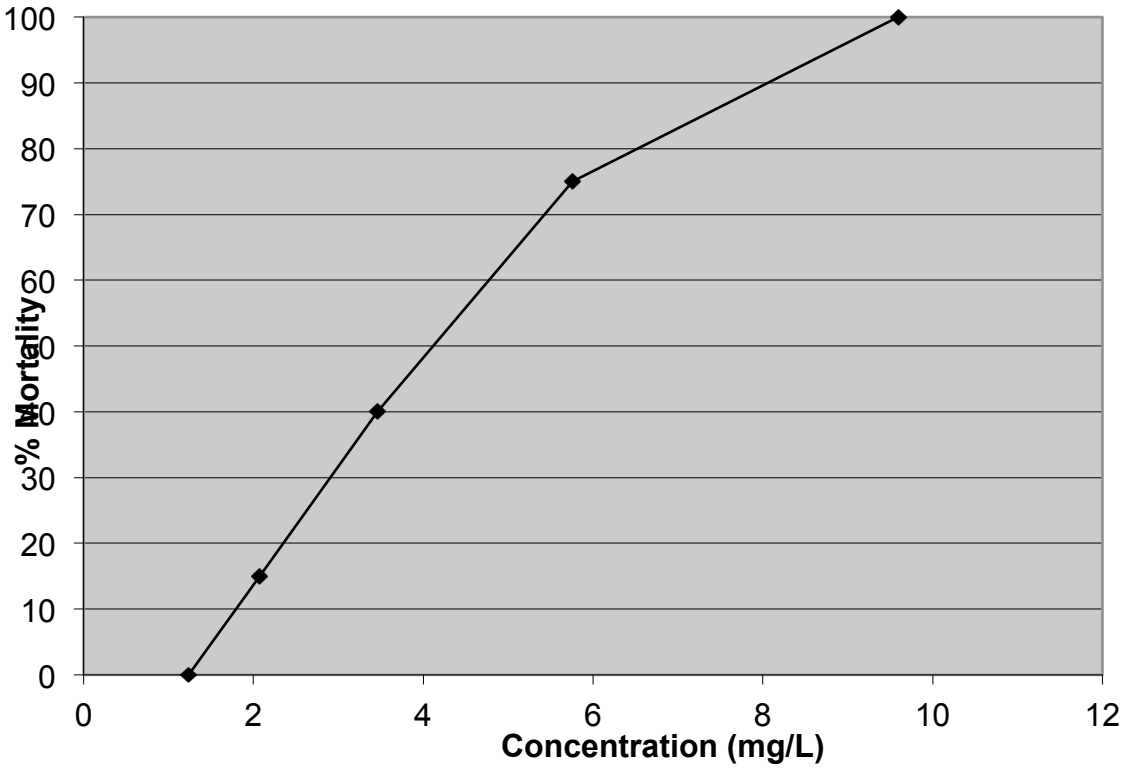
concentration. The EC50 value, based on growth rate inhibition, was calculated according to OECD Method 201.

5. **RESULTS**

5.1 Daphnia magna Acute Tests

Figure 5.1 graphically presents the results of the Daphnia magna acute test.

Figure 5.1. Concentration of BromMax 7.1 versus Daphnia magna Immobility



The Spearman-Karber method was applied to these data to obtain estimates of the 48 hour EC50 value and the associated 95% confidence interval. The results are as follows:

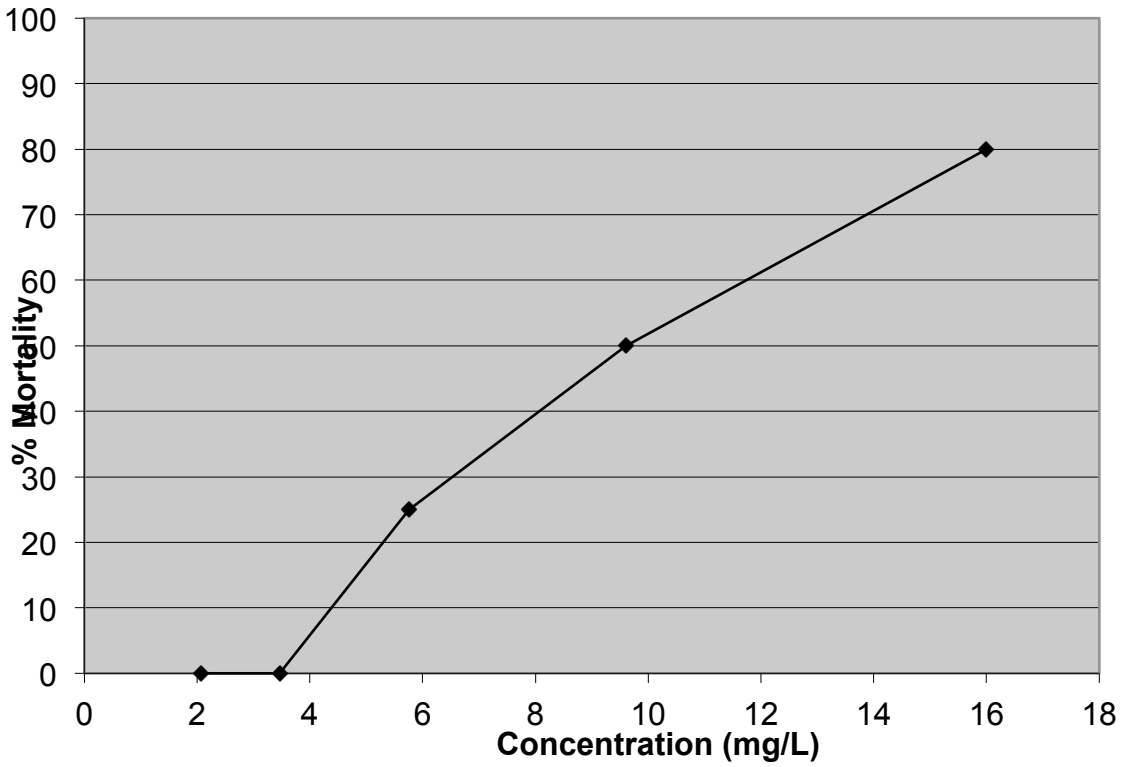
EC50 = 3.83 (3.23 – 4.54)

NOEC = 1.24 mg/L

5.2 Pimephales promelas Acute Test

Figure 5.2 graphically presents the results of the acute test with fathead minnows.

Figure 5.2 Concentration of BromMax 7.1 versus Fathead minnow Mortality



The Spearman-Karber method was applied to these data to obtain estimates of the 96 hour LC50 value and the associated 95% confidence interval. The results are as follows:

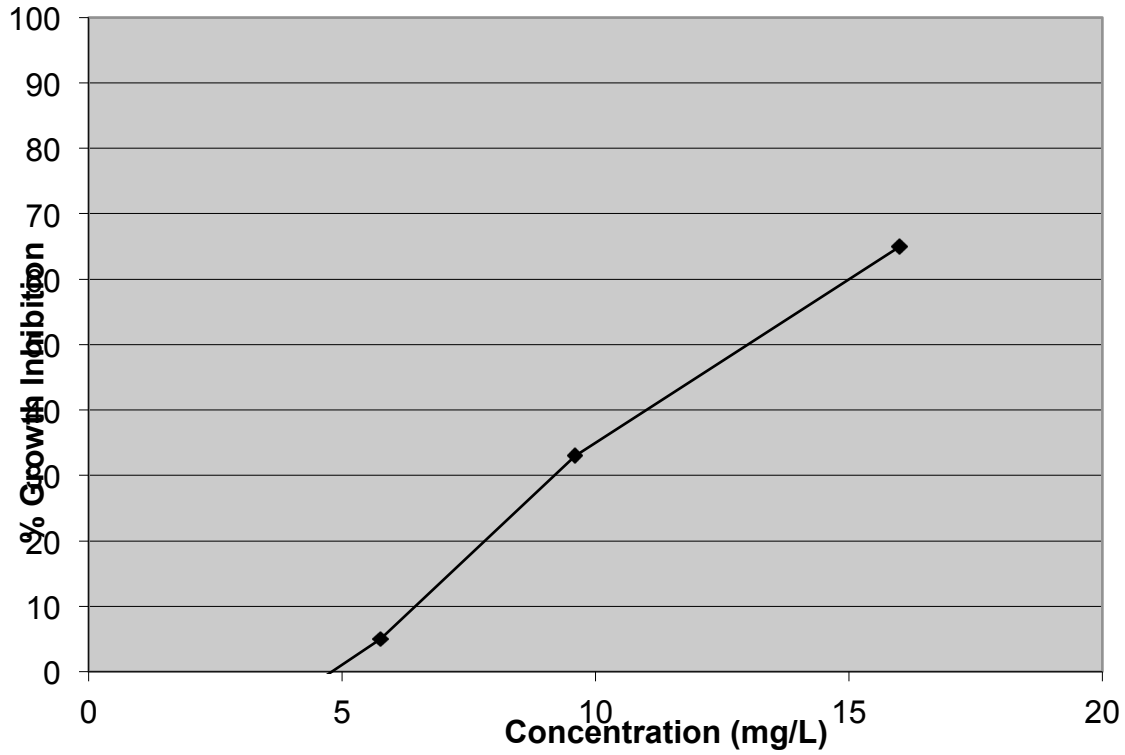
LC50 = 9.35 (7.41 – 11.8)

NOEC = 3.46

5.3 Selenastrum capricornutum Growth Inhibition Test

Figure 5.3 graphically presents the results of the algae growth inhibition test.

Figure 5.3 Concentration of BromMax 7.1 versus Algae Growth Inhibition



The EC50 was determined using a plot of the natural logarithm of concentration versus percent algal growth inhibition. The result was as follows:

EC50 = 13.4 mg/L

NOEC = 3.46 mg/L

6. **REFERENCES**

I.S.O. 1996. "Determination of the acute lethal toxicity of substances to a freshwater fish" (ISO 7346-2, 2nd edition, 1996).

O.E.C.D. 1984. OECD Guideline 201 for Testing of Chemicals - "Alga, Growth Inhibition Test". OECD 6/7/1984.

O.E.C.D. 1984. OECD Guideline 202 for Testing of Chemicals - "Daphnia sp., Acute Immobilisation Test and Reproduction Test". OECD 4/4/1984.

Stephan, C.E. (1977). Methods for calculating an LC50. In: Aquatic Toxicology and Hazard Evaluation (edited by F.I. Mayer and J.L. Hamelink). ASTM STP 634, pp 65-84, American Society for Testing and Materials.