



Nautilus Environmental, LLC

CI Agent Product Fathead Minnow Test Report

March 2005

Report date: April 6, 2005

Submitted to:

IRST
PO Box 7427
Louisville, KY 40857

Washington Laboratory
5009 Pacific Hwy East
Suite 2
Tacoma, WA 98424

1.0 INTRODUCTION

An acute toxicity rangefinding test was conducted using CI Agent Product submitted by IRST March 18 2005. The bioassay was conducted using the test organism *Pimephales promelas* (fathead minnow). Testing was performed at Nautilus Environmental's Northwest Laboratory located in Tacoma, Washington.

2.0 METHODS

2.1 Sample Transport and Receipt

The sample was transported to Nautilus by Brent Hepner. Appropriate chain-of-custody procedures were employed during transfer of the product to the laboratory.

2.2 Sample Preparation

Appropriate quantities of the sample were weighed and placed into each individual test chamber to achieve the target test concentrations. The product has a low solubility in water and, consequently, the majority of the sample remained on the surface of the test chambers throughout exposure, reducing the direct exposure to fathead minnows. The concentration of dissolved product in the test solutions is not known.

2.3 Test Methods

The acute toxicity test was conducted using fathead minnow according to procedures presented by USEPA (2002) and summarized in Table 1.

Table 1. Summary of test conditions for the 96h fathead minnow acute survival test.

Test initiation date and time	03/30/05; 1414h
Test termination date and time	04/03/05; 1335h
Test organism	<i>Pimephales promelas</i>
Test organism source	Aquatic BioSystems; Fort Collins, CO
Test organism age	5 days post hatch
Test duration	96 hours with solution renewal at 48 hours
Feeding	<i>Artemia</i> nauplii during holding time and 2 hours prior to solution renewal
Test chamber	250 mL plastic cup
Test solution volume	200 mL
Test temperature	20 ± 1°C
Dilution water	Moderately Hard Synthetic Water
Test concentrations (mg/L sample)	1000, 500, 100, 10, 1, control
Number of organisms/chamber	10
Number of replicates	4
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	EPA-821-R-02-012
Test acceptability criterion for controls	≥ 90% survival
Reference toxicant	Copper chloride

3.0 RESULTS

Survival was evaluated in the fathead minnow acute toxicity test after 96 hours of exposure. Results are summarized in Table 2. No adverse effects were observed in this test in any of the test concentrations. It should be noted that the concentrations of product presented in Table 2 do not reflect the dissolved concentration of material, and includes undissolved material present on the surface of the water in the test chambers.

Table 2. Summary of results for the acute toxicity tests.

Species	Concentration (mg/L)	Percent Survival	NOEC ^a (mg/L)	LOEC ^b (mg/L)	LC ₅₀ (mg/L)
<i>Pimephales promelas</i>	0	90	1000	>1000	>1000
	1	87.5			
	10	90			
	100	100			
	500	92.5			
	1000	90			

^a No Observed Effect Concentration, ^b Lowest Observed Effect Concentration

Individual statistical summaries for all tests and copies of the laboratory bench sheets are provided in Appendix A.

4.0 QA/QC

The sample was received in good condition. The toxicity test met acceptability criteria for performance of control organisms. There were no deviations from the protocol and water quality parameters remained within the ranges specified in the corresponding test method throughout the test.

A reference toxicant test used to monitor laboratory performance and test organism sensitivity is currently being conducted. Results for the most recently completed reference toxicant test are summarized in Table 3. The results for the reference toxicant test fell within the acceptable range of mean \pm two standard deviations of historical test results, indicating that the tests organisms were of an appropriate degree of sensitivity. The coefficient of variation (CV) for the test is also shown in the table.

Table 3. Reference toxicant test results.

Species	Date initiated	Endpoint	LC ₅₀ ($\mu\text{g/L}$ copper)	Historical mean \pm 2 SD ($\mu\text{g/L}$ copper)	CV (%)
Fathead minnow	03/02/05	96h survival	30.8	29.1 \pm 23.2	39.9

REFERENCES

- Tidepool Scientific Software. 2000-2003. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.025B.
- USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012.
- WDOE. 2001. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised December 2001.

Appendix A
Fathead Minnow Acute Toxicity Test
Statistical Summaries and Raw Bench Sheets

CETIS Test Summary

Report Date: 05 Apr-05 3:29 PM

Link: 11-7175-0375/0503-96NW

Fathead Minnow 96-h Acute Survival Test				Nautilus Environmental WA				
Test No:	02-5842-6942	Test Type:	Survival (96h)	Duration:	95h			
Start Date:	30 Mar-05 02:14 PM	Protocol:	EPA/821/R-02-012 (2002)	Species:	Pimephales promelas			
Ending Date:	03 Apr-05 01:35 PM	Dil Water:	Mod-Hard Synthetic Water	Source:	Aquatic Biosystems, CO			
Setup Date:	30 Mar-05 02:14 PM	Brine:						
Sample No:	08-8432-9998	Material:	Chemical Product	Client:				
Sample Date:	06 Mar-05	Code:	0503-96NW	Project:				
Receive Date:		Source:	IRST					
Sample Age:	24d 14h	Station:						
Comparison Summary								
Analysis	Endpoint	NOEL	LOEL	ChV	MSDp	Method		
10-1972-6262	96h Proportion Survived	1000	> 1000	N/A	18.91%	Dunnett's Multiple Comparison		
Point Estimate Summary								
Analysis	Endpoint	% Effect	Conc-mg/L	95% LCL	95% UCL	Method		
05-1778-8106	96h Proportion Survived	25	> 1000.00000	N/A	N/A	Linear Interpolation		
		50	> 1000.00000	N/A	N/A			
96h Proportion Survived Summary								
Conc-mg/L	Control Type	Reps	Mean	Minimum	Maximum	SE	SD	CV
0	Dilution Water	4	0.90000	0.80000	1.00000	0.05774	0.11547	12.83%
1		4	0.87500	0.70000	1.00000	0.06292	0.12583	14.38%
10		4	0.90000	0.80000	1.00000	0.04082	0.08165	9.07%
100		4	1.00000	1.00000	1.00000	0.00000	0.00000	0.00%
500		4	0.92500	0.80000	1.00000	0.04787	0.09574	10.35%
1000		4	0.90000	0.80000	1.00000	0.04082	0.08165	9.07%

CETIS Analysis Detail

Fathead Minnow 96-h Acute Survival Test Nautilus Environmental WA

Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
96h Proportion Survived	Comparison	11-7175-0375	11-7175-0375	05 Apr-05 3:27 PM	CETISv1.025

Method	Alt H	Data Transform	Z	NOEL	LOEL	Toxic Units	ChV	MSDp
Dunnett's Multiple Comparison	C > T	Angular (Corrected)		1000	>1000	0.10	N/A	18.91%

ANOVA Assumptions

Attribute	Test	Statistic	Critical	P Level	Decision(0.01)
Variances	Bartlett	7.67975	15.08628	0.17479	Equal Variances
Distribution	Shapiro-Wilk W	0.92923	0.88421	0.09871	Normal Distribution

ANOVA Table

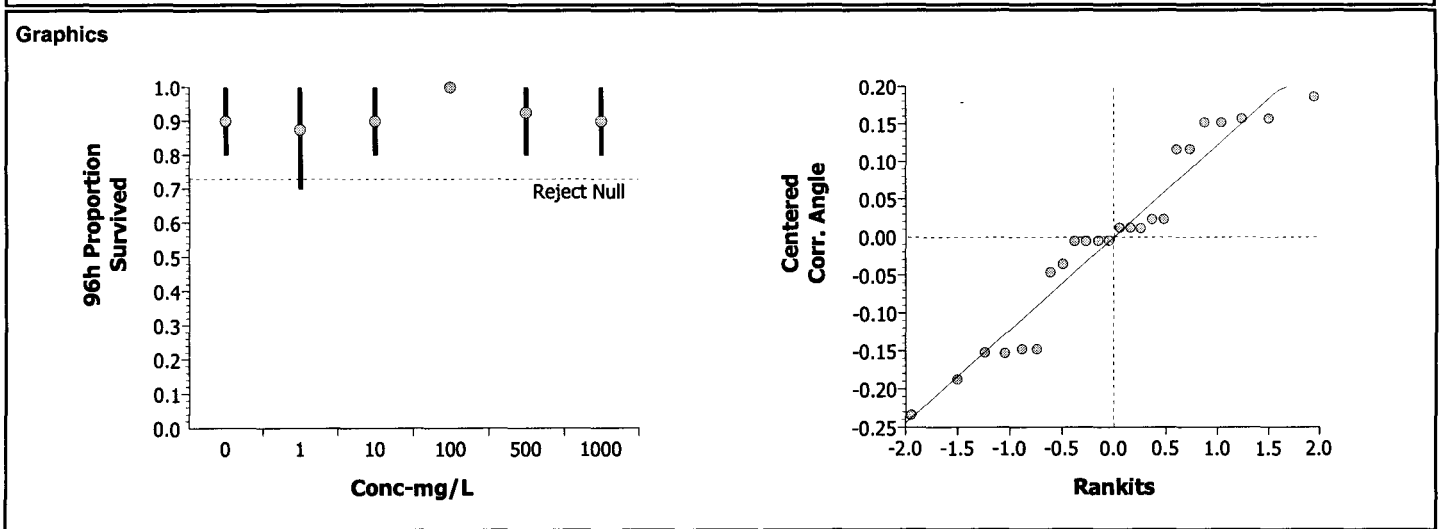
Source	Sum of Squares	Mean Square	DF	F Statistic	P Level	Decision(0.05)
Between	0.0776675	0.015534	5	0.81	0.55488	Non-Significant Effect
Error	0.3433514	0.019075	18			
Total	0.4210189	0.0346086	23			

Group Comparisons

Control	vs	Conc-mg/L	Statistic	Critical	P Level	MSD	Decision(0.05)
Dilution Water	1		0.35087	2.41	> 0.0500	0.23536	Non-Significant Effect
	10		0.05395	2.41	> 0.0500	0.23536	Non-Significant Effect
	100		-1.4411	2.41	> 0.0500	0.23536	Non-Significant Effect
	500		-0.3632	2.41	> 0.0500	0.23536	Non-Significant Effect
	1000		0.05395	2.41	> 0.0500	0.23536	Non-Significant Effect

Data Summary

Conc-mg/L	Control Type	Count	Original Data				Transformed Data			
			Mean	Minimum	Maximum	SD	Mean	Minimum	Maximum	SD
0	Dilution Water	4	0.90000	0.80000	1.00000	0.11547	1.25958	1.10715	1.41202	0.17602
1		4	0.87500	0.70000	1.00000	0.12583	1.22532	0.99116	1.41202	0.17399
10		4	0.90000	0.80000	1.00000	0.08165	1.25431	1.10715	1.41202	0.12461
100		4	1.00000	1.00000	1.00000	0.00000	1.40032	1.36523	1.41202	0.02340
500		4	0.92500	0.80000	1.00000	0.09574	1.29506	1.10715	1.41202	0.14695
1000		4	0.90000	0.80000	1.00000	0.08165	1.25431	1.10715	1.41202	0.12461



CETIS Analysis Detail

Linear Interpolation: Page 1 of 1
 Report Date: 05 Apr-05 3:29 PM
 Analysis: 05-1778-8106/0503-96NW

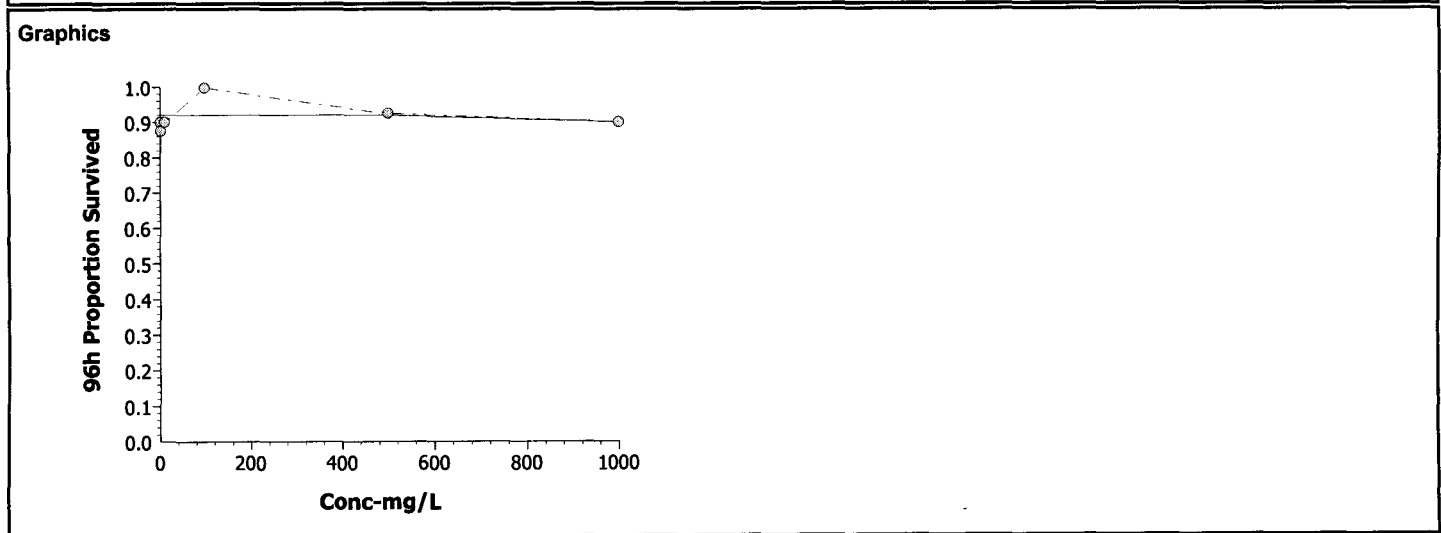
Fathead Minnow 96-h Acute Survival Test					Nautilus Environmental WA	
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Endpoint	Analysis Type	Sample Link	Control Link	Date Analyzed	Version
96h Proportion Survived	Linear Interpolation	11-7175-0375	11-7175-0375	05 Apr-05 3:28 PM	CETISv1.025

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Expanded CL	Method
Linear	Linear	7055475	200	Yes	Two-Point Interpolation

Point Estimates			
% Effect	Conc-mg/L	95% LCL	95% UCL
25	> 1000.00000	N/A	N/A
50	> 1000.00000	N/A	N/A

Data Summary		Calculated Variate(A/B)							
Conc-mg/	Control Type	Count	Mean	Minimum	Maximum	SE	SD	A	B
0	Dilution Water	4	0.90000	0.80000	1.00000	0.02357	0.11547	36	40
1		4	0.87500	0.70000	1.00000	0.02569	0.12583	35	40
10		4	0.90000	0.80000	1.00000	0.01667	0.08165	36	40
100		4	1.00000	1.00000	1.00000	0.00000	0.00000	36	36
500		4	0.92500	0.80000	1.00000	0.01954	0.09574	37	40
1000		4	0.90000	0.80000	1.00000	0.01667	0.08165	36	40



96 Hour Toxicity Test Data Sheet

Freshwater 96-hr Acute with Renewal

Client: WST
 Sample ID: C1 Agent
 Test #: 0503-9UNW

Start Date & Time: 3/30/05 1414
 End Date & Time: 04/03/05 1335
 Test Organism: P. promelas

mg/L Sample Conc. or %	D.O. (mg/L)			pH (mg/L)							
	Init.	Fin.	Init.	Fin.	Init.	Fin.					
0	24	48	48	72	96	0	24	48	48	72	96
Conc	8.5	8.5	8.0	8.6	8.5	7.5	8.00	7.84	7.94	8.01	7.90
1	8.5	8.5	8.0	8.2	8.4	7.5	7.98	7.95	8.04	8.12	8.09
10	8.6	8.5	8.2	8.5	8.5	7.6	8.00	7.99	8.08	8.08	7.97
100	8.4	8.4	8.0	8.0	8.5	7.6	8.10	8.00	8.09	8.12	8.07
500	8.4	8.6	8.5	8.1	8.4	7.5	8.10	8.03	8.04	8.13	8.04
1000	8.3	8.4	8.4	8.4	8.4	7.6	8.10	8.05	8.02	8.07	8.06

mg/L Sample Conc. or %	Conductivity (µS/cm)			Test Temperature (°C)					
	Init.	Fin.	Init.	Fin.	Init.	Fin.			
0	48	48	96	0	24	48	48	72	96
Conc	296	303	283	284	20.0	19.8	22.5	21.0	20.1
1	291	295	279	282	20.0	19.7	20.5	20.9	20.3
10	291	296	278	286	20.0	19.6	20.7	21.0	20.4
100	250	301	284	284	20.0	19.7	20.6	21.0	20.4
500	287	291	279	285	20.0	19.4	20.3	21.0	20.4
1000	286	294	276	281	20.0	19.6	20.3	21.0	20.3

Conc.	Alkalinity*	Hardness*	Chlorine Resid.	Ammonia
	(mg/L as CaCO3)	(mg/L)	(mg/L)	(mg/L)
control	60	80		
highest conc.	60	84		

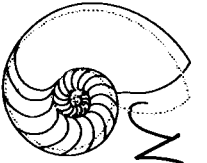
Comments: * cup of 18 dropped 3/31/05 Animal Source: ABS

Analysts: WJ JP Date Received: 3/30/05
Feed of 48 hrs Date of Hatch: 3/25/05

mg/L Sample Conc. or %	Rep #	Cont #	Number of Live Organisms				
			0	24	48	72	96
Conc	1	2	10	10	10	10	10
1	2	9	10	10	10	9	8
10	3	23	10	10	8	8	8
100	4	14	10	10	10	10	10
1000	1	11	10	10	10	10	10
500	2	3	10	10	10	9	7
100	3	22	10	10	9	9	9
10	4	6	10	10	9	9	9
10 mg/L	1	19	10	10	9	9	8
(2 mg)	2	24	10	10	10	9	9
100 mg/L	3	7	10	10	9	9	9
(20 mg)	4	15	10	10	10	10	10
500 mg/L	1	10	10	10	10	10	10
(100 mg)	2	13	10	10	10	10	10
1000 mg/L	3	20	10	10	10	10	10
(200 mg)	4	18	10	10	10	10	10
1000 mg/L	1	8	10	10	10	10	10
(100 mg)	2	1	10	10	10	10	10
1000 mg/L	3	13	10	10	10	10	10
(200 mg)	4	5	10	10	10	10	10
1000 mg/L	1	12	10	10	10	10	10
(200 mg)	2	21	10	10	9	9	9
1000 mg/L	3	4	10	10	9	8	8
(200 mg)	4	10	10	10	10	10	9

Tech. Initials: JP WJ CP AH AH

Appendix B
Chain-of-Custody Form



Nautilus Environmental, LLC

CALIFORNIA
5550 Morehouse Drive • Suite 150
San Diego, California 92121
Phone 858.587.7333
Fax 858.587.3961

WASHINGTON
5009 Pacific Highway East • Site 2
Tacoma, Washington 98424
Phone 253.922.4296
Fax 253.922.5814

Chain of Custody

Date 3/18 Page 1 of 1

Sample Collection by: Brent Hepner

Report to:

Company NextGen IRST
Address PO Box 7427
City Louisville State KY Zip 40257
Contact Dan Parker
Phone No. 1-502-247-0101

Invoice to:

Company Brent Hepner STL Labs
Address 5755 6th St. E.
City Tacoma State WA Zip 98672
Contact Brent Hepner
Phone No. 1-253-922-2300/cell 1-253-732-5919

SAMPLE ID

DATE

TIME

MATRIX

CONTAINER TYPE

NUMBER OF CONTAINERS

COMMENTS

RECEIPT TEMPERATURE (°C)

C1 Agent

3/6/05

1230

Sox. &

TR

Sox

without talc.

X

Furhead acute

PROJECT INFORMATION

SAMPLE RECEIPT

RELINQUISHED BY (CLIENT)

RELINQUISHED BY (COURIER)

CLIENT

TOTAL NO. OF CONTAINERS

(Signature) Brent Hepner

(Signature)

P.O. NO.

RECD GOOD CONDITION

(Printed Name) Brent Hepner

(Printed Name)

SHIPPED VIA:

MATCHES TEST SCHEDULE

(Company) STL Seattle NextGen

(Company)

SPECIAL INSTRUCTIONS/COMMENTS:

email to bhepner@stl-inc.com

RECEIVED BY (COURIER)

RECEIVED BY (LABORATORY)

Fax report to Brent when report complete

(Signature) [Signature]

(Time)

(Signature) [Signature]
Nautilus Environmental
1248 (Time)

253-922-5047

(Printed Name)

(Date)

(Printed Name) Monique I. L. 3/18/05
Nautilus Environmental Log-in No. 05-125
3/18/05 (Date)

and then mail to Louisville

(Company)

(Date)

Additional costs may be required for sample disposal or storage. Net 30 unless otherwise contracted.

DISTRIBUTION: WHITE - Nautilus Environmental, COLOR - Originator

DHEPNER@STL-inc.com