Study Title

Short-Term Chronic Toxicity of Salinity
To the Sheepshead Minnow (*Cyprinodon variegatus*)
Under Static-Renewal Test Conditions

Performed For

Parsons Environment & Infrastructure Group
9101 Burnet Road, Suite 210
Austin, TX 78758

Project Officer
Randy Palachek

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Study Period
18 June 2021 to 28 June 2021

Performing Laboratory

STILLMEADOW
INCORPORATED

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Certificate Number T104704352-20-13

Project Number
21-607-003

STATEMENT OF PROCEDURAL COMPLIANCE

I certify 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. The information contained herein is accurate and complete.

Janelle Mikulas, M.S.

06JUL21
Date

STATEMENT OF QUALITY ASSURANCE

The report and study data were audited to assure that the study was performed in accordance with STILLMEADOW, Inc. Standard Operating Procedures and regulatory guidelines. This report is an accurate reflection of the raw data.

Quality Assurance Auditor

07JUL21
Date

EXECUTIVE SUMMARY

Objective	The objective of this study was to determine the chronic toxicity of Salinity for Parsons Environment & Infrastructure Group to the Sheepshead Minnow, *Cyprinodon variegatus*.			
Study Director	Janelle Mikulas, M.S.			
Test Type	7-Day Static Renewal Short Term Chronic Toxicity Test			
Test Method	United States Environmental Protection Agency (EPA-821-R-02-014) (2002) Method 1006.0			
Test Dates (Times)	18 June 2021 (1054) to 25 June 2021 (1251)			
Test Substance	Salt			
Dilution Water	Synthetic Seawater			
Test Concentrations	Control (25 ppt), 30 ppt, 35 ppt, 40 ppt, 45 ppt			
Source of Organisms	STILLMEADOW Inc. Culture Laboratory			
Age of Test Organisms	7-11 days			
Test Acceptability	**Parameter**		**Test Data**	**EPA Criterion**
	Survival	Control	100%	≥80%
		Control CV[1]	0.00%	≤40%
		Highest Salinity CV	0.00%	≤40%
	Growth	Control	0.88 mg	≥0.50 mg
		Control CV	8.57%	≤40%
		Highest Salinity CV	10.13%	≤40%
		PMSD[2]	11.5	---
Test Results	**Parameter**		**Critical Concentration**	**NOEC[3] Test Solution**
	Survival		Pass	45 ppt
	Growth		Pass	45 ppt

[1]CV = Coefficient of Variation
[2]PMSD = Percent Minimum Significant Difference
[3]NOEC = No Observed Effect Concentration

INTRODUCTION

The objective of this study was to determine the chronic toxicity to *Cyprinodon variegatus* larvae of salinity for Parsons Environment & Infrastructure Group. This study is conducted in compliance with Texas Pollution Discharge Elimination System (TPDES) permit requirements; and in accordance with Texas Water Code Chapter 5, Subchapter R, Title 30 Texas Administrative Code Chapter 25 and the National Environmental Laboratory Accreditation Program (NELAP), Certificate Number T104704352-20-13. All original data, laboratory notebooks, and associated documentation are archived by the STILLMEADOW, Inc. Environmental Toxicology Laboratory.

METHODS AND MATERIALS

Test Substance/Dilution Water

Dilution water was synthetic seawater prepared according to USEPA (2002) guidelines. Initial characterization of the dilution and control water is given in Table 1. Dilution water was salted to the appropriate salinity for each test concentration.

Table 1. Chemical characterization of dilution water

Batch/Sample # Synthetic Seawater	Date Prepared	pH (SU)	Salinity (ppt)	Ammonia (mg/L NH_3N)	Total Residual Chlorine (mg/L)
QA21084	14 Jun 21	7.8	24	0.00	0.01
QA21086	17 Jun 21	7.8	26	0.00	0.02
QA21088	22 Jun 21	7.8	26	0.00	0.02

TEST CONDITIONS

The 7-day short-term chronic test using *Cyprinodon variegatus* and subsequent data analyses were carried out according to procedures specified by USEPA (2002) guidelines and STILLMEADOW, Inc. Environmental Toxicology Laboratory's Standard Operating Procedures. Table 2 lists a summary of the test conditions.

Table 2. Summary of test conditions

Organism lot #, Organism Source	AE210186, STILLMEADOW, Inc.
Organism age	7-11 days
Organisms per replicate	8
Replicates per concentration	5
Volume of test solution	500 mL
Test chamber	800-mL polystyrene beaker
Test temperature	25±1°C
Test duration	7 days
Dissolved oxygen	≥ 60% saturation
Photoperiod	16 L/ 8 D
Light intensity	50 – 100 ft c
Feeding regimen	twice daily, concentrated *Artemia* nauplii

Procedures

Test solutions (solutions for test renewals) were prepared daily at the STILLMEADOW, Inc. Environmental Toxicology Laboratory. The solutions were used for the renewals the day they were prepared.

Dissolved oxygen, salinity, pH, and temperature were measured in each treatment at the beginning and end of each 24-hour exposure period. Chamber temperature was also monitored daily. Aeration was not employed. The animals were fed twice daily during the test.

Test solutions were renewed by gently pouring old solutions out of the test beakers and replacing with new test solutions. During the renewal the larvae remained in the beaker along with approximately 20% of the old test solution.

At test initiation, at each renewal, and at test termination, the total number of live larvae was recorded for each test chamber. The unpreserved larvae from each test beaker were transferred to tared weigh boats at test termination and dried at 100-105°C for a minimum of 6 hours. The dried larvae were weighed to the nearest 0.001mg for determination of growth effects.

DATA ANALYSIS

All data were analyzed according to the statistical flow chart outlined in the EPA chronic testing manual (USEPA 2002). Table 3 lists the methods that were used in the analyses of the normality and homogeneity tests. A printout of statistical results is included in Appendix A.

TOXCALC™ Version 5.0 was used for all statistical evaluations. Survival and growth data were analyzed using hypothesis-testing techniques.

Table 3. Statistical methods used to analyze data for the toxicity test.

Endpoint	Comparison	Procedure
Survival	Transformation	Arc Sine $(y)^{1/2}$
	Normality	Shapiro-Wilk's Test ($\alpha \leq 0.01$)
	Homogeneity of Variances	Cannot Be Confirmed
	Reduction Relative to Control	Steel's Many-One Rank Test ($\alpha=0.05$)
Growth (Mean Dry Weight)	Transformation	No Transformation
	Normality	Shapiro-Wilk's Test ($\alpha \leq 0.01$)
	Homogeneity of Variances	Bartlett's Test ($\alpha \leq 0.01$)
	Reduction Relative to Control	Dunnett's Test ($\alpha=0.05$)

RESULTS

Survival and growth (mean dry weight) data for test organisms are provided in Table 4. Survival and mean dry weight at each concentration were compared to survival and weight of the control to determine statistically significant effects. The results of these comparisons are given in Table 5. Salinity over the course of the test is given in Table 6.

Table 4. Survival and mean dry weight for *M. beryllina* larvae exposed to test solutions for 7 days

Treatment (ppt)	Percent Survival (by day)				Dry Weight				Significant Effect Relative to Control	
					Original # of Fish		Surviving # of Fish			
	1	2	7	CV (%)	Mean (mg)	CV (%)	Mean (mg)	CV (%)	Survival	Mean Dry Weight[1]
25 (Control)	100	100	100	0.00	0.88	8.57	0.88	8.57		
30	100	100	100	0.00	0.95	7.72	0.95	7.72	NS[2]	NS
35	100	100	100	0.00	0.91	3.31	0.91	3.31	NS	NS
40	100	100	100	0.00	0.93	3.05	0.93	3.05	NS	NS
45	100	100	100	0.00	1.01	10.13	1.01	10.13	NS	NS

[1]Growth analysis for statistically significant effects relative to the control is based on the original number of fish.
[2]NS = Not Statistically Significant

Table 5. Summary of Statistical Endpoints.

Endpoint	Value (ppt)
Survival NOEC (No Observed Effect Concentration)	45
Growth NOEC	45

Table 6. Summary of Salinity.

Test Conc.	Salinity (parts per thousand)													
	Day 0	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7
	New	New	Old	New	Old	New	Old	New	Old	New	Old	New	Old	Old
25	25	25	26	25	26	25	22	25	26	25	26	24	24	24
30	30	30	30	30	30	30	28	29	31	30	31	29	29	29
35	35	35	35	35	34	36	32	34	36	35	36	34	34	34
40	40	40	40	40	40	41	38	39	41	40	41	39	39	39
45	44	44	44	44	44	45	41	44	46	44	46	44	44	44

REFERENCE TOXICANT TEST RESULTS

STILLMEADOW, Inc. conducts routine standard reference toxicant testing using *Cyprinodon variegatus* obtained from STILLMEADOW, Inc. cultures. Sodium Dodecyl Sulfate (SDS) is used as the reference toxicant with synthetic seawater as the dilution water; the test method followed is USEPA method 1006.0 (USEPA, 2002). A copy of STILLMEADOW, Inc.'s most recent standard reference toxicant control chart for this species is presented in Appendix B.

STUDY DEVIATIONS

No deviations from the prescribed guidelines or standard operating procedures were identified during the study.

REFERENCES

U.S. Environmental Protection Agency (USEPA). 2002. *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. Third Edition, October 2002. EPA-821-R-02-014.

Ives, Michael A. TOXCALC™ Version 5.0. 1994. TidePool Scientific Software. McKinleyville, California.

APPENDIX A

Statistical Analysis

Larval Fish Growth and Survival Test-7 Day Survival

Start Date:	6/18/2021	Test ID:	21-607-003	Sample ID:	salt
End Date:	6/25/2021	Lab ID:	QA21084/86/88	Sample Type:	
Sample Date:		Protocol:	EPA-821-R-02-014	Test Species:	CV-Cyprinodon variegatus
Comments:					

Conc-ppt	1	2	3	4	5
ontrol (25 ppt)	1.0000	1.0000	1.0000	1.0000	1.0000
30	1.0000	1.0000	1.0000	1.0000	1.0000
35	1.0000	1.0000	1.0000	1.0000	1.0000
40	1.0000	1.0000	1.0000	1.0000	1.0000
45	1.0000	1.0000	1.0000	1.0000	1.0000

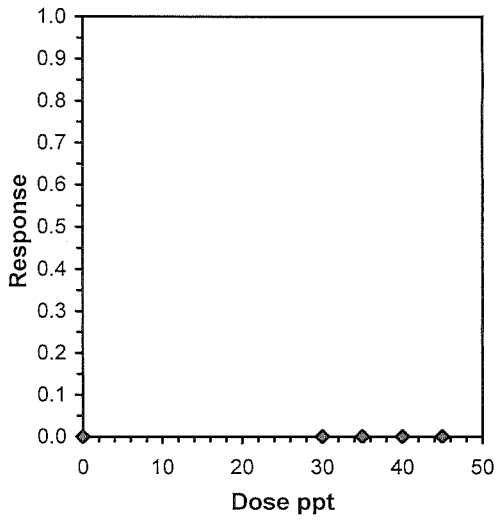
			Transform: Arcsin Square Root					Rank	1-Tailed	Isotonic	
Conc-ppt	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
ontrol (25 ppt)	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5			1.0000	1.0000
30	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000
35	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000
40	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000
45	1.0000	1.0000	1.3931	1.3931	1.3931	0.000	5	27.50	17.00	1.0000	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.888		
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	45	>45		

Linear Interpolation (200 Resamples)

Point	ppt	SD	95% CL(Exp)	Skew
IC05	>45			
IC10	>45			
IC15	>45			
IC20	>45			
IC25	>45			
IC40	>45			
IC50	>45			



Reviewed by: 3/24/22

Larval Fish Growth and Survival Test-7 Day Biomass

Start Date:	6/18/2021	Test ID:	21-607-003	Sample ID:	salt
End Date:	6/25/2021	Lab ID:	QA21084/86/88	Sample Type:	
Sample Date:		Protocol:	EPA-821-R-02-014	Test Species:	CV-Cyprinodon variegatus
Comments:					

Conc-ppt	1	2	3	4	5
ontrol (25 ppt)	0.9584	0.9155	0.8964	0.8815	0.7569
30	0.9911	0.9179	1.0063	0.8319	0.9896
35	0.9231	0.8664	0.9008	0.8964	0.9463
40	0.9819	0.9348	0.9231	0.9170	0.9105
45	1.0476	0.8809	1.1299	1.0508	0.9233

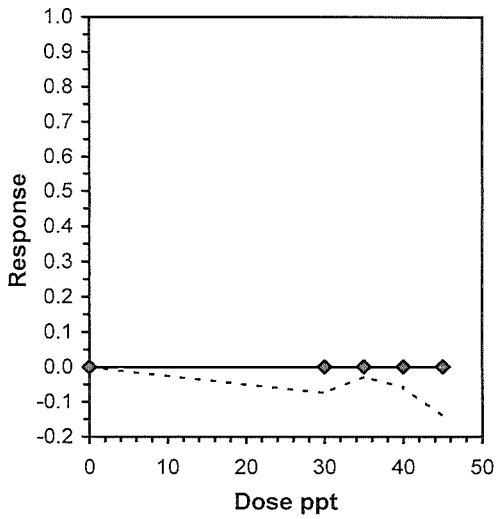
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ppt	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
ontrol (25 ppt)	0.8817	1.0000	0.8817	0.7569	0.9584	8.566	5				0.9351	1.0000
30	0.9474	1.0744	0.9474	0.8319	1.0063	7.717	5	-1.525	2.300	0.0990	0.9351	1.0000
35	0.9066	1.0282	0.9066	0.8664	0.9463	3.310	5	-0.577	2.300	0.0990	0.9351	1.0000
40	0.9335	1.0587	0.9335	0.9105	0.9819	3.054	5	-1.202	2.300	0.0990	0.9351	1.0000
45	1.0065	1.1415	1.0065	0.8809	1.1299	10.130	5	-2.898	2.300	0.0990	0.9351	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93518	0.888	-0.582	0.25992
Bartlett's Test indicates equal variances (p = 0.09)	8.06551	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	45	>45			0.099	0.11228	0.01114	0.00463	0.08371	4, 20

Linear Interpolation (200 Resamples)

Point	ppt	SD	95% CL(Exp)	Skew
IC05	>45			
IC10	>45			
IC15	>45			
IC20	>45			
IC25	>45			
IC40	>45			
IC50	>45			



Reviewed by: JM 3/Jun/21

APPENDIX B

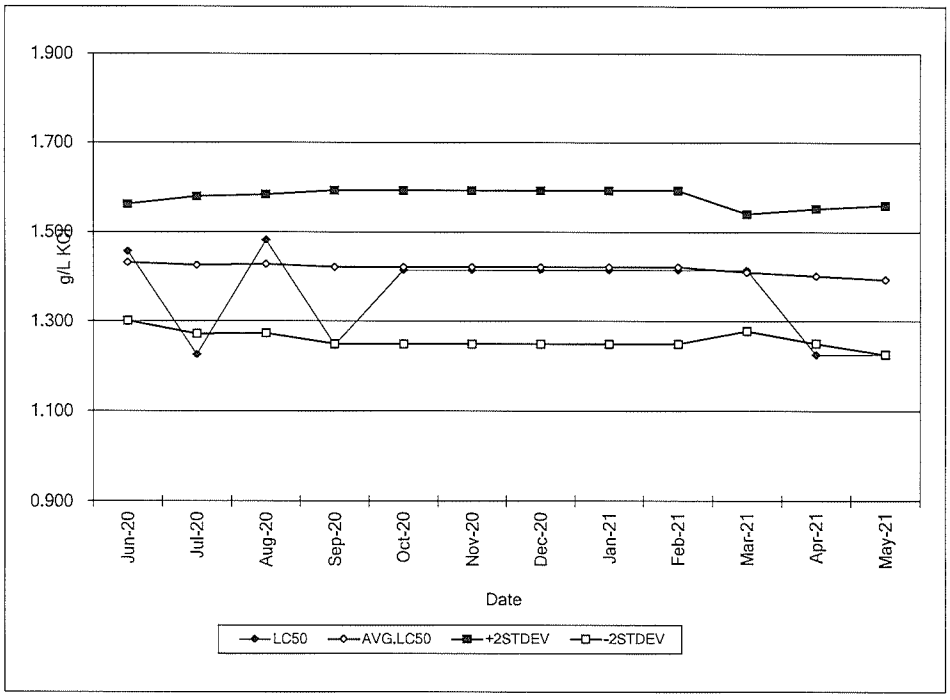
Standard Reference Toxicant Control Charts

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel:970/484-5091 Fax:970/484-2514

REFERENCE TOXICANT LC50

Cyprinodon variegatus



48 HOUR ACUTE TOXICITY DATA FOR

Cyprinodon variegatus

DATE	LC50 (g/L KCl)	95% CONFIDENCE (upper)	(lower)	AVG.LC50 (g/L KCl)	METHOD	Avg+2std	Avg-2std
Jul 20	1.457	1.577	1.345	1.431	SPKR	1.562	1.301
Aug 20	1.225	1.225	1.225	1.425	SPKR	1.579	1.271
Sep 20	1.482	1.604	1.369	1.428	SPKR	1.583	1.273
Oct 20	1.246	1.290	1.204	1.421	SPKR	1.593	1.249
Nov 20	1.414	1.414	1.414	1.421	Graphical	1.593	1.249
Dec 20	1.414	1.414	1.414	1.421	Graphical	1.593	1.249
Jan 21	1.414	1.414	1.414	1.421	Graphical	1.593	1.249
Feb 21	1.414	1.414	1.414	1.421	Graphical	1.593	1.249
Mar 21	1.414	1.414	1.414	1.421	Graphical	1.593	1.249
Apr 21	1.414	1.414	1.414	1.409	Graphical	1.541	1.278
May 21	1.225	1.225	1.225	1.401	Graphical	1.552	1.250
Jun 21	1.225	1.225	1.225	1.393	Graphical	1.560	1.225

Current Test Dates:6/28-30/2021