Rainier ENVIRONMENTAL

Whole Effluent Toxicity Test Report: Washington Beef LLC.

March 2013

Report date: April 9, 2013

Submitted to:

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1.0 INTRODUCTION

A whole effluent toxicity test was conducted using effluent samples collected from the Washington Beef LLC wastewater treatment plant in March 2013. A chronic bioassay was conducted using the test organism *Ceriodaphnia dubia* (*Ceriodaphnia*). Testing was performed at Rainier Environmental Laboratory located in Tacoma, Washington.

2.0 METHODS

2.1 Sample Collection and Transport

Effluent samples were collected into 4-liter (L) LDPE cubitainers by Washington Beef personnel. The samples were packed in coolers containing ice and shipped to Rainier Environmental by overnight delivery service. Appropriate chain-of-custody procedures were employed during collection and transport (Appendix D).

2.2 Sample Receipt

Upon arrival at the laboratory, coolers were opened, samples inspected, and the contents verified against information provided on the chain-of-custody forms. Receipt temperature was measured and recorded on the chain-of-custody form. The standard water quality parameters were measured and recorded on sample check-in sheets (Appendix B). Samples were stored at 4°C in the dark until used for testing.

2.3 Test Methods

A chronic toxicity test was conducted according to procedures presented by USEPA (2002). The methods are summarized in Table 1. The procedure involved a 7-day static-renewal exposure to the effluent. The endpoints from these tests were *Ceriodaphnia* survival at the end of exposure and reproduction at test termination or production of 3 broods, whichever occurred first. Termination of the test occurred when at least 60 percent of surviving control females produced 3 broods. The test was ended on Day 7.

Table 1. Summary of methods for the 7-day *Ceriodaphnia* survival and reproduction test.

Test initiation date and time	3/26/13; 1500h
Test termination date and time	4/2/13; 1445h
Test organism	*Ceriodaphnia dubia*
Test organism source	In-house cultures
Test organism age	< 24 hours
Test duration	7 days; Test terminated when 60% of controls reached 3 broods
Feeding	1:1 mixture YTC:algal suspension daily
Test chamber; test solution volume	30 mL plastic cup; 15 mL
Test temperature	25 ± 1°C
Dilution water	Diluted mineral water
Test concentrations (% sample)	100, 50, 25, 12.5, 6.25, laboratory control
Number of organisms/chamber	1
Number of replicates	10
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	EPA-821-R-02-013
Test acceptability criteria for controls	≥ 80% survival; ≥ 15 neonates per surviving adult
Reference toxicant	Sodium chloride

3.0 RESULTS AND DISCUSSION

Details of standard water quality measurements conducted upon receipt of samples are provided in Table 2.

Table 2. Final Effluent sample information.

Parameter	WET		
Rainier Log-in No.	13-034	12-035	12-036
Collection date and time	3/25/2013; 0715h	3/27/2013; 0730h	3/29/2013; 0920h
Receipt date and time	3/26/2013; 1000h	3/28/2013; 1400h	3/30/2013; 0930h
Receipt temperature (°C)	0.7	0.3	2.0
Dissolved oxygen (mg/L)	8.4	9.1	8.7
pH	7.97	7.88	7.86
Conductivity (µS/cm)	4850	3990	4160
Salinity (ppt)	2.4	2.1	2.2
Hardness (mg/L $CaCO_3$)	80	64	68
Alkalinity (mg/L $CaCO_3$)	152	120	124
Total Chlorine (mg/L) [a]	<0.03	<0.03	<0.03
Total Ammonia (mg/L) [b]	<1.0	<1.0	<1.0

[a,b] See reference below

Note: Total chlorine and ammonia values are measured by Rainier Environmental to provide additional information in support of the bioassay test procedures. They are not intended to be interpreted as exact values, particularly near the detection limits where interferences are most likely to become apparent.

[a] Total chlorine is measured using a Hach DR/2000 spectrophotometer and colorimetric DPD Total Chlorine Reagent. Under optimum conditions, the method has a range of 0.03 to 2.0 mg/L ± 0.01 mg/L total chlorine. Compounds in the sample that interfere with chlorine detection include bromine, manganese, chromium, ozone, and peroxides. Additional interferences include extreme pH values and high alkalinity (greater than 300 mg/L Ca CO_3).

[b] Total ammonia is measured using a Hach DR/2000 spectrophotometer following the salicylate method which uses AmVer Diluent Reagent Test 'N Tube kits. Under optimum conditions, the method has a range of 0.4 to 50.0 ± 0.1 mg/L NH_3-N. High sample turbidity will give erroneously high values. Additional interferences to the method include extreme pH and high concentrations of magnesium, iron, nitrite, nitrate, or sulfate.

Results for the toxicity tests are summarized in Table 3. Individual statistical summaries for the test and copies of the laboratory bench sheets are provided in the Appendices A-D.

The NOEC (concentration at which no effect on the organisms is detected) was 100 percent sample for survival and 50 percent for reproduction. The associated chronic toxicity unit (TUc; 100 percent sample divided by the NOEC) was 1 for survival and 2 for reproduction.

Table 3. Summary of toxicity test results.

Sample	Endpoint	NOEC (% effluent)	Chronic Toxicity Unit (TUc) [a]
Final Effluent	Survival	100	1.0
	7-day Reproduction	50	2.0

[a] Chronic toxicity unit (TU_c = 100 ÷ NOEC)

4.0 QA/QC

Samples were received in good condition and within the temperature range specified by EPA (2002). The toxicity tests met all acceptability criteria for performance of control organisms. There were no deviations from protocol and water quality parameters remained within the ranges specified in the corresponding test methods throughout the tests.

Results for the most recent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 4 and Appendix C. The coefficients of variation (CV) for the endpoints are also shown in the table. The results for the reference toxicant test fell within the acceptable range of mean ± two standard deviations of historical test results indicating that the test organisms were of an appropriate degree of sensitivity.

Table 4. Reference toxicant test results.

Species	Endpoint	Date initiated	LC_{50}/EC_{50}	Acceptable Range	CV (%)
Ceriodaphnia	7d survival	3/12/2013	2.30 g/L NaCl	1.27 – 2.84 g/L	22.4
	7d reproduction	3/12/2013	1.53 g/L NaCl	1.07 - 1.54 g/L	9.5

REFERENCES

Tidepool Scientific Software. 2000-2010. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.0.8.

USEPA. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. pp. 141-196.

Appendix A
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Test Code: 1303-032 | 09-7979-7435

Ceriodaphnia 7-d Survival and Reproduction Test — **Rainier Environmental Laboratory**

Batch ID:	01-4488-3861	**Test Type:**	Reproduction-Survival (7d)	**Analyst:**	Eric Tollefson
Start Date:	26 Mar-13 15:00	**Protocol:**	EPA/821/R-02-013 (2002)	**Diluent:**	Perrier Water
Ending Date:	02 Apr-13 14:45	**Species:**	Ceriodaphnia dubia	**Brine:**	
Duration:	7d	**Source:**	In-House Culture	**Age:**	<24h

Sample ID:	18-9322-9568	**Code:**	13-034	**Client:**	Washington Beef
Sample Date:	25 Mar-13 07:15	**Material:**	POTW Effluent	**Project:**	
Receive Date:	26 Mar-13 10:00	**Source:**	Washington Beef (WA0050202)		
Sample Age:	32h (0.7 °C)	**Station:**			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-8450-5891	7d Survival Rate	100	>100	NA	NA	1	Fisher Exact/Bonferroni-Holm Test
14-2952-7186	Reproduction	50	100	70.71	24.9%	2	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-6238-1610	7d Survival Rate	LC5	50	15.8	59.5	2	Linear Interpolation (ICPIN)
		LC10	55.23	19.9	100	1.811	
		LC15	60.99	50	N/A	1.639	
		LC20	67.35	57.47	N/A	1.485	
		LC25	74.36	61.6	N/A	1.345	
		LC40	100	74.36	N/A	1	
		LC50	>100	N/A	N/A	<1	
17-6032-6224	Reproduction	IC5	51.21	16.03	52.91	1.953	Linear Interpolation (ICPIN)
		IC10	53.87	20.48	56.15	1.856	
		IC15	56.67	50.57	59.49	1.765	
		IC20	59.61	53.79	63.03	1.678	
		IC25	62.7	57.02	67.14	1.595	
		IC40	72.94	66.95	81.1	1.371	
		IC50	80.67	73.3	91.63	1.24	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-6238-1610	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
18-8450-5891	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria
14-2952-7186	Reproduction	Control Resp	27.4	15 - NL	Yes	Passes Acceptability Criteria
17-6032-6224	Reproduction	Control Resp	27.4	15 - NL	Yes	Passes Acceptability Criteria
14-2952-7186	Reproduction	PMSD	0.2494	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
6.25		10	1	1	1	1	1	0	0	0.0%	0.0%
12.5		10	1	1	1	1	1	0	0	0.0%	0.0%
25		10	0.9	0.7819	1	0	1	0.1	0.3162	35.14%	10.0%
50		10	1	1	1	1	1	0	0	0.0%	0.0%
100		10	0.6	0.4072	0.7928	0	1	0.1633	0.5164	86.07%	40.0%

Reproduction Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	27.4	26.5	28.3	24	32	0.763	2.413	8.81%	0.0%
6.25		10	37.7	36.52	38.88	32	41	1.001	3.164	8.39%	-37.59%
12.5		10	35.5	34.06	36.94	30	42	1.222	3.866	10.89%	-29.56%
25		10	31.7	27.41	35.99	0	39	3.63	11.48	36.21%	-15.69%
50		10	33.6	32.85	34.35	31	37	0.636	2.011	5.99%	-22.63%
100		10	9.6	5.853	13.35	0	25	3.174	10.04	104.5%	64.96%

Ceriodaphnia 7-d Survival and Reproduction Test — Rainier Environmental Laboratory

7d Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
6.25		1	1	1	1	1	1	1	1	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	0	1	1
50		1	1	1	1	1	1	1	1	1	1
100		1	1	0	1	1	1	0	0	0	1

Reproduction Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	25	28	28	32	24	26	25	28	29	29
6.25		41	41	38	32	38	38	40	33	40	36
12.5		36	41	33	42	35	32	35	33	30	38
25		35	38	36	33	31	33	39	0	33	39
50		35	33	33	37	31	34	32	31	36	34
100		3	25	0	14	14	21	0	0	0	19

7d Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
6.25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
12.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
50		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
100		1/1	1/1	0/1	1/1	1/1	1/1	0/1	0/1	0/1	1/1

Rainier Environmental
Washington Laboratory

Client: Washington Beef
Sample ID. WET
Test No: 1303-032
Log-In#: 13-034 13-035 13-036

Initial and Final Chemistries
Seven Day Chronic Freshwater Bioassay
Start Date & Time: 3/26/13 1500
Stop Date & Time: 4/2/13 1445
Test Species: Ceriodaphnia dubia

Conc. or % CON	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.94	8.01	7.85	7.95	7.78	7.97	7.81	7.97	7.92	8.02	8.01	7.95	7.95	7.93
DO (mg/l)	7.8	8.1	7.7	8.1	7.8	8.1	8.0	8.1	7.9	8.1	8.0	7.9	7.9	8.0
Cond. (µmhos-cm)	~~1850~~	203	192	205	191	202	187	201	187	193	188	192	190	193
Temperature (°C)	24.6	25.5	25.1	25.4	25.0	25.2	25.2	25.4	25.0	25.5	25.1	25.4	25.0	25.5

6.25	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.92	8.03	7.86	7.99	7.79	7.98	7.81	7.99	7.87	8.01	7.92	7.97	7.96	7.97
DO (mg/l)	7.9	8.1	7.7	8.2	7.9	8.1	7.9	8.1	7.8	8.1	8.0	8.0	7.9	7.8
Cond. (µmhos-cm)	613	628	615	622	592	607	522	553	524	535	518	525	521	515
Temperature (°C)	24.8	25.5	25.1	25.4	24.8	25.2	25.1	25.4	25.0	25.5	25.2	25.4	25.0	25.5

12.5	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.87	8.01	7.86	8.02	7.81	8.01	7.83	8.00	7.84	8.03	7.85	7.97	7.92	8.00
DO (mg/l)	7.9	8.2	7.8	8.2	8.0	8.1	7.9	8.2	7.8	8.1	7.8	8.0	7.9	7.9
Cond. (µmhos-cm)	905	915	903	935	865	903	767	813	781	807	752	772	750	762
Temperature (°C)	25.0	25.5	25.2	25.4	24.8	25.2	25.1	25.4	25.1	25.5	25.2	25.4	25.1	25.5

25	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.85	8.05	7.83	8.05	7.78	8.00	7.75	8.02	7.75	8.03	7.77	8.01	7.85	8.02
DO (mg/l)	8.0	8.1	7.8	8.0	8.0	8.0	7.9	8.2	8.0	8.2	7.9	8.2	8.0	8.0
Cond. (µmhos-cm)	1462	1476	1465	1483	1334	1375	1223	1257	1237	1262	1192	1215	1185	1192
Temperature (°C)	25.0	25.5	25.2	25.4	25.0	25.2	25.2	25.4	25.2	25.5	25.1	25.4	25.1	25.5

50	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.78	8.07	7.75	8.07	7.73	8.04	7.72	8.05	7.72	8.08	7.71	8.05	7.81	8.06
DO (mg/l)	8.1	8.2	7.8	8.2	7.9	8.1	7.9	8.1	7.9	8.1	7.8	8.1	8.0	8.2
Cond. (µmhos-cm)	~~2585~~	2623	2592	2597	2611	2605	2285	2355	2297	2313	2238	2258	2241	2263
Temperature (°C)	25.3	25.5	25.2	25.4	25.1	25.2	25.2	25.4	25.4	25.5	25.2	25.4	25.0	25.5

100	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.73	8.11	7.72	8.10	7.68	8.05	7.65	8.07	7.69	8.12	7.70	8.10	7.78	8.08
DO (mg/l)	8.0	8.2	7.8	8.2	7.9	8.1	7.9	8.2	8.0	8.2	7.9	8.2	8.0	8.2
Cond. (µmhos-cm)	4780	4820	4670	4710	4510	4520	4020	4110	4080	4120	3990	4020	4070	4110
Temperature (°C)	25.8	25.5	25.2	25.4	25.2	25.2	25.4	25.4	25.7	25.5	25.3	25.4	25.0	25.5
Tech. Initials	ef	ef	ef	ef	ef	ef	ef	ef	ef	ef	ef	ef	ef	ef

Dilution Water Batch #: DMW 002
Test Chamber: VWR

QA Check: ef

Sample Description:
Animal Source: In House Culture
Date Received: —
Date of Hatch: —
Comments:

Ceriodaphnia 7-Day Chronic Survival and Reproduction

Client/Sample ID: Washington Beef / WET
Test Number: 1303-032

Start Date and Time: 3/26/13 1500
Stop Date and Time: 4/2/13 1445

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	CON	47	—	—	—	2	6	—	17		8	25
2		4	—	—	—	3	7	—	18		10	28
3		33	—	—	—	3	7	—	18		10	28
4		46	—	—	—	4	8	—	20		12	32
5		11	—	—	—	3	6	—	15		9	24
6		2	—	—	—	3	7	—	16		10	26
7		20	—	—	—	3	7	—	15		10	25
8		43	—	—	—	4	10	—	14		14	28
9		38	—	—	—	3	11	—	15		14	29
10		28	—	—	—	4	10	—	15		14	29
Analyst			RT	RT	RT	RT	RT	RT	RT			
Time		1500	1100	1500	1000	855	1030	1345	1445			
Selen #		002	002	002	002	002	002	002	002			

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	25	44	—	—	—	7	11	—	17		18	35
2		30	—	—	—	8	12	—	18		20	38
3		57	—	—	—	7	12	17	—		36	36
4		15	—	—	—	6	11	16	—		33	33
5		17	—	—	—	5	11	—	15		16	31
6		37	—	—	—	6	11	—	16		17	33
7		60	—	—	—	8	15	16	—		39	39
8		55	—	—	—	X					0	0
9		1	—	—	—	8	12	13	—		33	33
10		45	—	—	—	8	13	—	18		21	39

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	6.25	7	—	—	—	7	15	—	19		22	41
2		6	—	—	—	7	13	—	21		20	41
3		34	—	—	—	8	14	16	—		38	38
4		10	—	—	—	6	11	15	—		32	32
5		27	—	—	—	7	12	—	19		19	38
6		19	—	—	—	6	13	—	19		19	38
7		26	—	—	—	8	14	—	18		22	40
8		40	—	—	—	6	11	16	—		33	33
9		35	—	—	—	6	14	20	—		40	40
10		56	—	—	—	7	11	—	18		18	36

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	50	8	—	—	—	6	13	—	16		19	35
2		18	—	—	—	6	12	—	15		18	33
3		24	—	—	—	6	12	15	—		33	33
4		54	—	—	—	7	13	17	—		37	37
5		50	—	—	—	5	11	—	15		16	31
6		9	—	—	—	7	11	—	16		18	34
7		3	—	—	—	7	10	15	—		32	32
8		53	—	—	—	6	11	14	—		31	31
9		32	—	—	—	5	13	18	—		36	36
10		29	—	—	—	7	12	15	—		34	34

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	12.5	41	—	—	—	6	11	—	19		17	36
2		14	—	—	—	7	15	—	19		22	41
3		22	—	—	—	5	12	16	—		33	33
4		13	—	—	—	6	15	21	—		42	42
5		5	—	—	—	6	11	—	18		17	35
6		31	—	—	—	6	10	—	16		16	32
7		51	—	—	—	7	12	16	—		35	35
8		39	—	—	—	5	12	16	—		33	33
9		48	—	—	—	6	10	14	—		30	30
10		42	—	—	—	7	14	17	—		38	38

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	100	23	—	—	—	3	—	—	—		3	3
2		21	—	—	—	3	—	7	15		10	25
3		16	—	—	X						0	0
4		49	—	—	—	2	4	8	—		14	14
5		58	—	—	—	2	—	2	10		4	14
6		52	—	—	—	4	—	8	9		12	21
7		59	—	—	—	X					0	0
8		12	—	—	X						0	0
9		36	—	—	X						0	0
10		25	—	—	—	2	—	3	14		5	19

Comments: X=mortality

QA RT

Appendix B
Sample Check-In Sheets

Rainier Environmental
5013 Pacific Hwy East, Ste. 20
Tacoma, WA 98424

Sample Check-In Information

Client: Washington Beef

Tests Performed: Cd-C

Test ID No(s).: 1303-032

Sample Description: Brown

Sample ID:	WET	WET	Final Effluent Outfall 002	
Log-in No. (10-xxxx):	13-034	13-035	13-036	
Sample Collection Date & Time:	3/25/13 715	3/27/13 730	3/29/13 920	
Sample Receipt Date & Time:	3/26/13 1000	3/28/13 1400	3/30/13 930	
Check-in Temperature (°C)	0.7	0.3	2.0	
Temperature OK?	(Y) N	(Y) N	(Y) N	Y N
DO (mg/L)	8.4	9.1	8.7	
pH (units)	7.97	7.88	7.86	
Conductivity (µS/cm)	4850	3990	4160	
Salinity (ppt)	2.4	2.1	2.2	
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	3.8 / 25 / 152	3.0 / 25 / 120	3.1 / 25 / 124	/ /
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* [a]	2.0 / 25 / 80	1.6 / 25 / 64	1.7 / 25 / 68	/ /
Total Chlorine (mg/L)	<0.03	<0.03	<0.03	
Total Ammonia (mg/L)	<1.0	<1.0	<1.0	
Technician Initials	ET	ET	ET	

* = mg/L as $CaCO_3$, [a] = Measured for freshwater samples only, NA = Not Applicable, NM = Not Measured

COC Complete? Y or N
1 Y 2 Y 3 Y

Filtration? Y (N)
Pore Size: _____
Organisms or Debris

Aeration? Y (N)
Length of Time: _____
Final DO: _____
Final pH: _____

Freshwater Tests:

Control/Dilution Water Source: test type: Cd-C (8:2 (DMW)) 002 MHW Other: _____ Alkalinity: 64 Hardness: 84

Control/Dilution Water Source: test type: _____ 8:2 (DMW) MHW Other: _____ Alkalinity: _____ Hardness: _____

Additional Control? Y N = _____ Alkalinity: _____ Hardness: _____

Hardness Adjustment? Y (N)
If adjusted, please see worksheet for details.

Marine Tests:

Control/Dilution Water Source: test type: _____ ART SW NAT SW Alkalinity: _____ Salinity: _____

Control/Dilution Water Source: test type: _____ ART SW NAT SW Alkalinity: _____ Salinity: _____

Additional Control? Y N = _____ Alkalinity: _____ Salinity: _____

Sample Salted w/ artificial salt? Y N If yes, what ppt? _____ test type: _____

Sample salted w/brine? Y N If yes, what ppt? _____ test type: _____

Sub-samples for additional chemistry:

Comments: Temperature for grab sample must be 0-20°C if received within 1 hour of collection time, 0-12°C if effluent received within 4 hours of collection time, and 0-6°C for all other samples.

QC Check: ET

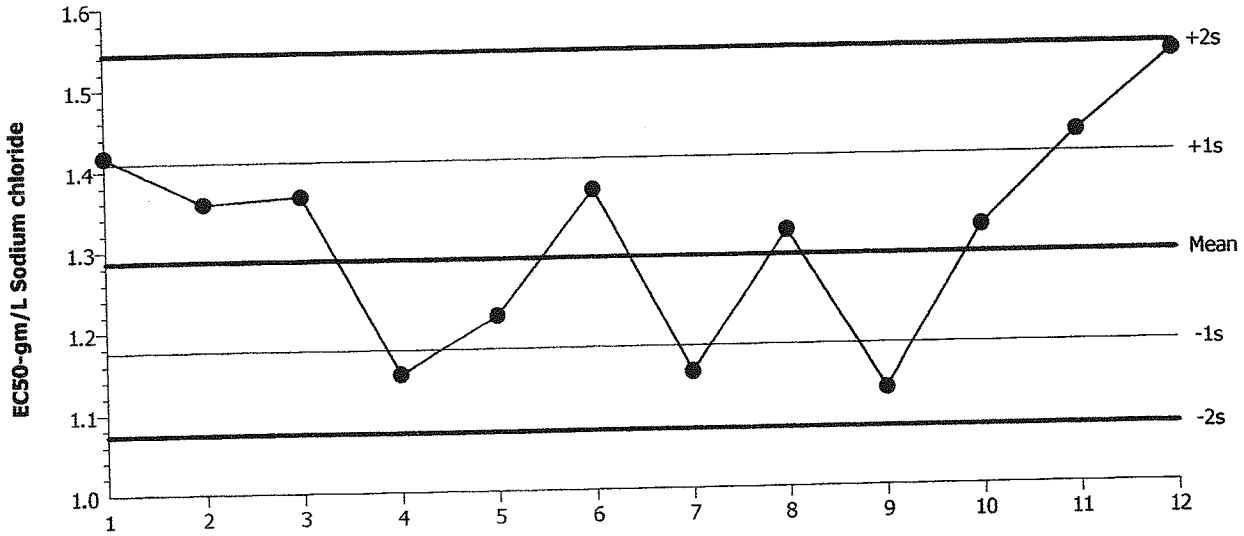
Appendix C
Reference Toxicant Test

CETIS QC Plot

Ceriodaphnia 7-d Survival and Reproduction Test — **Rainier Environmental Laboratory**

Test Type: Reproduction-Survival (7d) **Organism:** Ceriodaphnia dubia (Water Flea) **Material:** Sodium chloride
Protocol: EPA/821/R-02-013 (2002) **Endpoint:** Reproduction **Source:** Reference Toxicant-REF

Ceriodaphnia 7-d Survival and Reproduction Test



Mean:	1.287	**Count:**	11	**-1s Warning Limit:**	1.175	**-2s Action Limit:**	1.073
Sigma:	NA	**CV:**	9.50%	**+1s Warning Limit:**	1.409	**+2s Action Limit:**	1.543

Quality Control Data

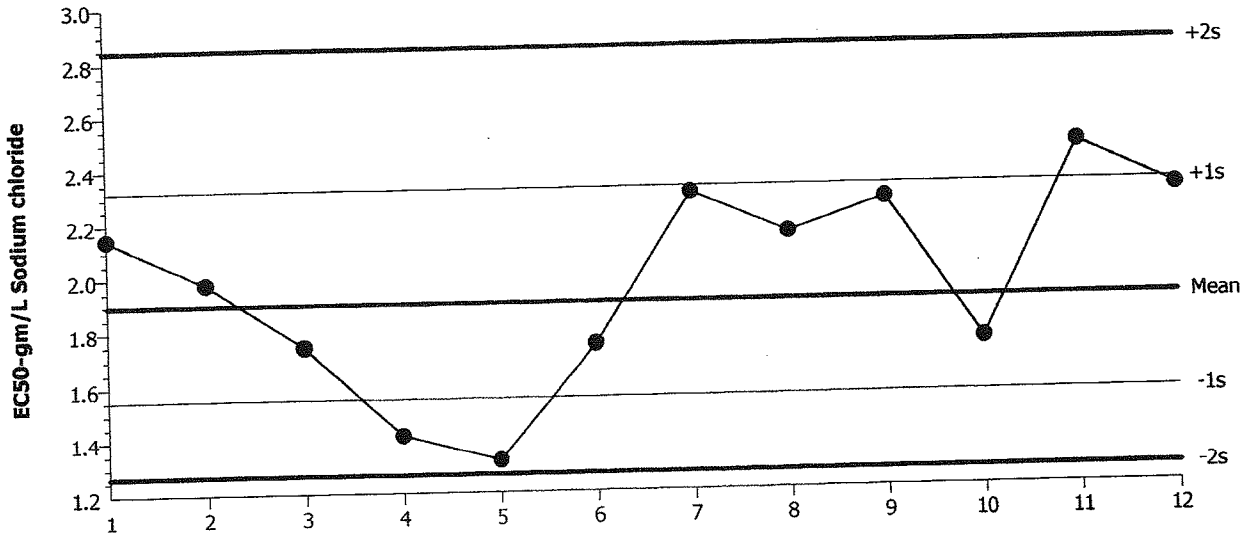
Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Nov	10	1.417	0.1304	1.064	(+)		18-1646-5148	02-8001-5569
2			18	1.358	0.07104	0.5921			11-7820-4865	14-3007-5816
3			19	1.366	0.07891	0.6558			08-1813-0818	00-3657-8188
4			25	1.146	-0.1408	-1.277	(-)		12-3676-9409	18-2334-7627
5		Dec	2	1.216	-0.0705	-0.6209			03-1806-9758	11-1567-8111
6	2012	Mar	20	1.37	0.08326	0.6909			04-4181-4802	14-9819-7042
7		Jun	26	1.144	-0.1431	-1.299	(-)		18-3335-1100	06-3063-9294
8		Jul	17	1.316	0.02928	0.2479			02-9547-9197	03-2978-8518
9		Sep	18	1.121	-0.1663	-1.525	(-)		20-3257-9401	21-2717-5233
10		Oct	30	1.319	0.03248	0.2747			14-7011-9138	13-0927-4963
11		Dec	11	1.435	0.1483	1.202	(+)		18-7111-4230	00-5310-2281
12	2013	Mar	12	1.533	0.2461	1.928	(+)		13-9507-4728	19-0889-8785

CETIS QC Plot

Ceriodaphnia 7-d Survival and Reproduction Test — **Rainier Environmental Laboratory**

Test Type:	Reproduction-Survival (7d)	**Organism:**	Ceriodaphnia dubia (Water Flea)	**Material:**	Sodium chloride
Protocol:	EPA/821/R-02-013 (2002)	**Endpoint:**	7d Survival Rate	**Source:**	Reference Toxicant-REF

Ceriodaphnia 7-d Survival and Reproduction Test



Mean:	1.897	**Count:**	11	**-1s Warning Limit:**	1.55	**-2s Action Limit:**	1.267
Sigma:	NA	**CV:**	22.40%	**+1s Warning Limit:**	2.322	**+2s Action Limit:**	2.842

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Nov	10	2.144	0.2461	0.6038			18-1646-5148	10-8016-4521
2			18	1.977	0.0796	0.2034			11-7820-4865	00-4433-4101
3			19	1.741	-0.1563	-0.4257			08-1813-0818	01-3437-6850
4			25	1.414	-0.4832	-1.455	(-)		12-3676-9409	20-8924-6879
5		Dec	2	1.32	-0.5779	-1.798	(-)		03-1806-9758	03-8081-7233
6	2012	Mar	20	1.741	-0.1563	-0.4257			04-4181-4802	06-8447-2630
7		Jun	26	2.297	0.4	0.9469			18-3335-1100	10-1106-6325
8		Jul	17	2.144	0.2461	0.6038			02-9547-9197	16-5989-6607
9		Sep	18	2.267	0.3692	0.8801			20-3257-9401	11-6459-7205
10		Oct	30	1.741	-0.1563	-0.4257			14-7011-9138	01-0378-8759
11		Dec	11	2.462	0.5649	1.29	(+)		18-7111-4230	17-4716-3730
12	2013	Mar	12	2.297	0.4	0.9469			13-9507-4728	11-5347-8239

Rainier Environmental
Washington Laboratory

Client: Reference Toxicant
Sample ID. 4g/L NaCl
Test No: RT03/2/13CD
Log-In#: —

Initial and Final Chemistries
Seven Day Chronic Freshwater Bioassay
Start Date & Time: 3/12/13 1615
Stop Date & Time: 3/19/13 1630
Test Species: Ceriodaphnia dubia

Conc. or %	Days													
	0		1		2		3		4		5		6	
CON	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.72	8.01	7.85	8.00	7.90	8.02	7.92	7.95	7.85	8.01	7.84	7.92	7.92	7.95
DO (mg/l)	8.1	8.2	7.9	8.1	8.0	8.0	7.9	8.2	8.0	8.1	8.0	8.2	7.9	8.1
Cond. (µmhos-cm)	182	201	185	189	187	203	181	205	185	192	187	195	183	191
Temperature (°C)	24.5	25.3	25.1	25.5	24.8	25.3	24.5	25.5	25.1	25.2	25.0	25.5	24.7	25.2

0.25	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.73	8.05	7.87	7.98	7.91	8.05	7.81	7.97	7.87	8.01	7.82	7.95	7.91	7.97
DO (mg/l)	8.1	8.2	7.9	8.1	8.0	8.1	7.9	8.2	7.8	8.1	8.0	8.1	8.0	8.2
Cond. (µmhos-cm)	658	671	655	662	648	672	660	668	662	671	671	677	662	673
Temperature (°C)	24.5	25.3	25.0	25.5	24.8	25.4	24.5	25.5	25.1	25.2	24.9	25.5	24.7	25.2

0.5	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.75	8.00	7.89	8.03	7.91	8.01	7.81	7.98	7.91	7.98	7.85	7.97	7.89	7.95
DO (mg/l)	8.2	8.2	8.0	8.2	7.9	8.1	7.8	8.2	7.8	8.2	7.8	8.0	8.1	8.2
Cond. (µmhos-cm)	1167	1175	1168	1202	1152	1213	1177	1210	1182	1208	1191	1212	1172	1185
Temperature (°C)	24.5	25.3	25.0	25.5	25.2	25.4	24.5	25.5	25.0	25.3	24.9	25.5	24.6	25.2

1.0	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.75	7.95	7.88	8.03	7.92	8.01	7.83	8.01	7.93	8.03	7.85	8.01	7.90	7.99
DO (mg/l)	8.2	8.2	8.0	8.2	7.9	8.0	7.8	8.2	7.9	8.2	7.9	8.0	8.1	8.2
Cond. (µmhos-cm)	2133	2147	2123	2185	2108	2207	2187	2205	2154	2193	2147	2215	2140	2157
Temperature (°C)	24.5	25.3	25.0	25.5	25.1	25.4	~~25.0~~ 24.5	25.5	25.0	25.3	24.9	25.5	24.6	25.2

2.0	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.74	8.03	7.91	8.04	7.91	8.02	7.84	8.03	7.95	8.04	7.86	8.03	7.91	8.00
DO (mg/l)	8.1	8.2	7.9	8.2	7.9	8.0	7.8	8.2	7.9	8.2	7.9	8.2	8.0	8.2
Cond. (µmhos-cm)	3990	4020	4010	4110	3970	4200	4100	4110	3980	4000	4110	4170	3990	4020
Temperature (°C)	24.5	25.3	24.9	25.5	25.1	25.4	24.5	25.5	25.0	25.3	24.9	25.5	24.7	25.2

4.0	Days													
	0		1		2		3		4		5		6	
	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final	init.	final
pH	7.74	8.04												
DO (mg/l)	8.1	8.2												
Cond. (µmhos-cm)	7570	7620												
Temperature (°C)	24.5	25.3												
Tech. Initials	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT

Dilution Water Batch #: DMW001
Test Chamber: VWR

QA Check: CT

Sample Description:
Animal Source: In House Culture
Date Received: —
Date of Hatch: —
Comments:

Ceriodaphnia 7-Day Chronic Survival and Reproduction

Client/Sample ID: Reference Toxicant / 4g/L NaCl
Test Number: RT031213CD

Start Date and Time: 3/12/13 1615
Stop Date and Time: 3/19/13 1630

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	CON	19	—	—	—	6	—	15	21		21	42
2		27	—	—	—	6	—	12	15		18	33
3		29	—	—	—	5	—	14	17		19	36
4		57	—	—	—	—	5	12	—		17	17
5		3	—	—	—	5	—	15	—		20	20
6		48	—	—	—	7	—	16	—		23	23
7		49	—	—	—	8	—	16	18		24	42
8		54	—	—	—	5	—	15	20		20	20
9		15	—	—	—	6	—	14	22		20	42
10		10	—	—	—	8	—	17	23		25	48
Analyst		QA	QA	QA	QA	QA	QA	QA	QA			
Time		1615	1500	1515	1430	1230	1330	1400	1630			
Selen #		002	002	002	002	002	002	002	002			

Rep	Conc.	Cont	Daily Reproduction 1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	1.0	51	—	—	—	5	—	13	22		18	40
2		11	—	—	—	7	—	12	24		19	43
3		52	—	—	—	7	—	15	20		22	42
4		33	—	—	—	—	4	10	—		14	14
5		41	—	—	—	4	—	17	—		21	21
6		13	—	—	—	8	—	16	—		24	24
7		23	—	—	—	7	—	15	21		22	43
8		45	—	—	—	6	—	14	20		20	40
9		42	—	—	—	5	—	14	19		19	38
10		34	—	—	—	6	—	12	17		18	35

Rep	Conc.	Cont	1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	0.25	30	—	—	—	5	—	12	20		17	37
2		44	—	—	—	6	—	14	18		20	38
3		37	—	—	—	6	—	14	18		20	38
4		16	—	—	—	—	4	10	—		14	14
5		1	—	—	—	4	—	13	—		17	17
6		7	—	—	—	7	—	15	—		22	22
7		50	—	—	—	8	—	17	23		25	48
8		46	—	—	—	8	—	14	18		24	42
9		59	—	—	—	7	—	12	19		19	38
10		8	—	—	—	5	—	15	14		20	39

Rep	Conc.	Cont	1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	2.0	36	—	—	—	—	3	5	—		8	8
2		5	—	—	—	—	2	5	—		7	7
3		26	—	—	—	X	—	—	—		0	0
4		56	—	—	—	—	—	2	3		2	5
5		4	—	—	—	—	7	4	—		11	11
6		39	—	—	—	X	—	—	—		0	0
7		17	—	—	—	—	4	3	—		7	7
8		20	—	X	—	—	—	—	—		0	0
9		21	—	—	—	—	—	—	—		0	0
10		22	—	—	—	—	5	4	—		9	9

Rep	Conc.	Cont	1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	0.5	38	—	—	—	6	—	13	20		19	39
2		32	—	—	—	5	—	14	22		19	41
3		53	—	—	—	7	—	15	24		22	46
4		55	—	—	—	—	5	11	—		16	16
5		58	—	—	—	8	—	17	—		25	25
6		12	—	—	—	7	—	15	—		22	22
7		14	—	—	—	5	—	14	19		19	38
8		18	—	—	—	5	—	12	18		17	35
9		2	—	—	—	4	—	11	17		15	32
10		43	—	—	—	4	—	13	17		17	34

Rep	Conc.	Cont	1	2	3	4	5	6	7	8	Day 6 Total	Third Brood
1	4.0	28	X	—	—	—	—	—	—	—	0	0
2		31	X	—	—	—	—	—	—	—	0	0
3		35	X	—	—	—	—	—	—	—	0	0
4		25	X	—	—	—	—	—	—	—	0	0
5		47	X	—	—	—	—	—	—	—	0	0
6		6	X	—	—	—	—	—	—	—	0	0
7		9	X	—	—	—	—	—	—	—	0	0
8		24	X	—	—	—	—	—	—	—	0	0
9		40	X	—	—	—	—	—	—	—	0	0
10		60	X	—	—	—	—	—	—	—	0	0

Comments: X=mortality

QA QA

Appendix D
Chain-of-Custody Forms

Chain of Custody

Rainier ENVIRONMENTAL

Washington
5013 Pacific Highway East, Suite 20 Fife,
WA 98424
Phone253.922.8898

Date 3/25/13 Page___of___

Sample Collection By:

Report to:

Company	Washington Beef LLC
Address	201 Elmwood ~~Wate~~ Rd
City/State/Zip	Toppenish WA. 98948
Contact	Sherry Byers
Phone	509-952-6534
Email	Sherry.Byers@abfoodsusa.com

Invoice To:

Company	
Address	Same
City/State/Zip	
Contact	
Phone	
Email	

ANALYSES REQUIRED

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	WET										Receipt Temperature (°C)
1	WET	3/25/13	7:15 am	Waste Water	Cubitainer	1		X										0.7
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		

PROJECT INFORMATION

Client:	
PO No.:	
Shipped Via:	UPS

SAMPLE RECEIPT

Total No. of Containers	1
Received Good Condition?	Y
Matches Test Schedule?	Y

SPECIAL INSTRUCTIONS/COMMENTS:

RELINQUISHED BY (CLIENT)

(Signature) (Time) 10:20am

(Printed Name) (Date) Annette M.H. Richardson 3/25/13

(Company) Washington Beef LLC.

RELINQUISHED BY (COURIER)

(Signature) (Time)

(Printed Name) (Date)

(Company)

RECEIVED BY (COURIER)

(Signature) (Time)

(Printed Name) (Date)

(Company)

RECEIVED BY (LABORATORY)

(Signature) Eric Tollefson (Time) 1000

(Printed Name) ERIC TOLLEFSON (Date) 3/26/13

(Log In #) 13-034

Chain of Custody

Rainier ENVIRONMENTAL

Washington
5013 Pacific Highway East, Suite 20 Fife,
WA 98424
Phone253.922.8898

Date 3/27/13 Page ___ of ___

Sample Collection By:

Report to:

Company	Washington Beef LLC
Address	201 Elmwood Rd.
City/State/Zip	Toppinish WA. 98948
Contact	Sherry Byers
Phone	509-952-6534
Email	Sherry.Byers@abfoods usa.com

Invoice To:

Company	
Address	
City/State/Zip	Same
Contact	
Phone	509-865-2121
Email	

ANALYSES REQUIRED

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	WET	Receipt Temperature (°C)
1	WET	3/27/13	7:30am	waste water	Cubitainer	1		X	0.3
2									
3									
4									
5									
6									
7									
8									
9									
10									

PROJECT INFORMATION

Client:	
PO No.:	
Shipped Via:	UPS

SAMPLE RECEIPT

Total No. of Containers	1
Received Good Condition?	Y
Matches Test Schedule?	Y

SPECIAL INSTRUCTIONS/COMMENTS:

RELINQUISHED BY (CLIENT)

(Signature) [signature] (Time) 10:30AM
(Printed Name) Annette M Harris-Richardson (Date) 3/27/13
(Company) Washington Beef LLC

RELINQUISHED BY (COURIER)

(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (COURIER)

(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (LABORATORY)

(Signature) Eric Tollefson (Time) 3/28/13
(Printed Name) ERIC TOLLEFSON (Date) 1400
(Log In #) 13-035

Chain of Custody

Rainier ENVIRONMENTAL

Washington
5013 Pacific Highway East, Suite 20 Fife,
WA 98424
Phone253.922.8898

Date_____Page___of___

Sample Collection By:

Report to:

Company	Washington Beef LLC
Address	201 Elmwood Rd
City/State/Zip	Toppenish, WA. 98948
Contact	Sherry Byers-Eddy
Phone	(509) 865-2121
Email	Sherry.Byers@abfoodusa.com

Invoice To:

Company	Washington Beef LLC
Address	201 Elmwood Rd
City/State/Zip	Toppenish, WA. 98948
Contact	Sherry Byers-Eddy
Phone	(509) 865-2121
Email	Sherry.Byers@abfoodusa.com

	SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	ANALYSES REQUIRED: WET	Receipt Temperature (°C)
1	Final Effluent Outfall 002	3/29/13	9:00am	Wastewater	1 gallon cubitainer	1		✓	2.0
2									
3									
4									
5									
6									
7									
8									
9									
10									

PROJECT INFORMATION

Client:	
PO No.:	
Shipped Via:	UPS

SAMPLE RECEIPT

Total No. of Containers	1
Received Good Condition?	Y
Matches Test Schedule?	Y

SPECIAL INSTRUCTIONS/COMMENTS:

RELINQUISHED BY (CLIENT)

(Signature) Mike H (Time) 7:25am
(Printed Name) Mike Hernandez (Date) 3/29/30
(Company) Washington Beef LLC.

RECEIVED BY (COURIER)

(Signature) (Time)
(Printed Name) (Date)
(Company)

RELINQUISHED BY (COURIER)

(Signature) (Time)
(Printed Name) (Date)
(Company)

RECEIVED BY (LABORATORY)

(Signature) Eric Tollefson (Time) 930
(Printed Name) ERIC TOLLEFSON (Date) 3/30/13
(Log in #) 13-036